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PEOPLE AND EVENTS

UNIVERSITY NEWS

Frank B. Morrison Hall Dedicated at Cornell

Frank B. Morrison Hall, Cornell University's $4,500,000 animal husbandry building, was dedicated September 12 and 13.

As keynote speaker, New York GOVERNOR NELSON A. ROCKEFELLER said the building was dedicated "not only to the memory of an outstanding man, not only to agricultural progress, but to a better future for mankind."

The new building, of natural brick and limestone, contains over 300 rooms in approximately 133,000 sq. ft. of floor space. Included are offices; six lecture rooms, with one seating 314; five teaching laboratories; 20 research laboratories; two conference rooms; small-animal quarters; and special rooms for X-ray studies, small- and large-animal surgery, microbiological assays, and electronic data processing. A seminar room, reading room, and library are located on the building's third floor.

Of special interest in the meat studies area is the modern slaughter floor, a carbon dioxide hog immobilizer for humane slaughtering, a $10,000 temperature-humidity controlled smokehouse, and a meat sales room.

Rockefeller and other speakers on the dedication program paid tribute to the late professor of animal husbandry and animal nutrition for whom the building was named. DR. FRANK B. MORRISON, who came to Cornell in 1927 and was head of the Animal Husbandry Department for 16 yr., is known throughout the world for his book Feeds and Feeding. The prologue to the Spanish edition calls it "the most important work in Agriculture published in the present century." Dr. Morrison's wife and his two sons, Dr. Roger Morrison and Dr. Spencer Morrison, attended the dedication ceremonies.

In addition to Gov. Rockefeller, speakers on the dedication program included: DR. THOMAS H. HAMILTON, president of the State University of New York; DR. CHARLES E. PALM, dean of the New York State College of Agriculture; DON J. WICKHAM, commissioner of the New York State Department of Agriculture and Markets; E. P. FORRESTEL, chairman of the Livestock Advisory Council to the Department of Animal Husbandry; ALBERT K. MITCHELL, Cornell University trustee and prominent New Mexico cattleman; and DR. KENNETH L. TURK, head of Cornell's Animal Husbandry Department. Cornell PRESIDENT DEANE W. MALOTT presided at the afternoon session with the REV. MR. WALTER A. DODDS, First Presbyterian Church, Ithaca, N. Y., giving the invocation.

DR. JAMES H. HILTON, president of Iowa State University, was the featured speaker at a dedication dinner in Cornell's Statler Hall on the evening of September 13. Discussing The Animal Industry in a Period of Change, Dr. Hilton called upon the Land-Grant colleges and universities to meet the challenge of the world population explosion.

DR. WILLIAM I. MYERS, former dean of the College of Agriculture, presided at the evening meeting, and guests were introduced by Dr. Turk.

Climaxing the two-day dedication period was a morning symposium of special interest to members of the animal industry entitled, Animal Nutrition's Contributions to Modern Animal Agriculture.

The five symposium speakers included: DR. FREDERICK N. ANDREWS, professor and head of the Department of Dairy Husbandry at Purdue University; DR. L. A. MOORE, Dairy Cattle Research Branch, U. S. Department of Agriculture; DR. LEONARD A. MAYNARD, Cornell professor emeritus of biochemistry and nutrition; DR. THOMAS J. REID, Cornell professor of animal husbandry; and DR. JOHN K. LOOSLI, Cornell professor of animal nutrition. Presiding was DR. JOHN I. MILLER, Cornell professor of animal husbandry.

All five talks summarized past research achievements and stressed the need for further scientific investigations if animal husbandry is to continue its present growth.

Connecticut Researchers Awarded Grants

B. W. PICKETT of the Department of Animal Industries, University of Connecticut, has received a $39,675 grant from the National Institutes of Health to study Lipid Composition of Mammalian Semen. R. G. JENSEN has received $23,000 to continue studies on Lipase Specificity.

South Carolina News

Clemson Agricultural College, Clemson, South Carolina, held its annual Farm and Home Week, August 14-18. Numerous exhibits were on display on Bowman field.

The Dairy Science Program consisted of a series of talks on a variety of subjects, such as: Coastal Bermuda pastures, total solids in the pricing of milk, D.H.I.A. records, milk production of crossbred dairy cows, and the use of pelleted roughages for the feeding of dairy cattle and farm animals in general. Artificial

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insemination, practices and its future, was discussed at one of the afternoon sessions. DR. H. A. HERMAN, Executive Secretary, National Association of Artificial Breeders, Columbia, Missouri, was the featured speaker for this group. In addition to staff members, the following guest speakers participated in the program: T. C. BLALOCK, Extension Specialist in Charge of Dairy Extension, N. C. State, Raleigh, N. C.; C. R. HUSTON, Assistant Manager, Field Services Division, Noba, Inc., Tiffin, Ohio.

Various demonstrations were conducted on mechanical feeding of silage, sire selection, branding, dehorning, and others. Registered attendance for these meetings was 125.

Ohio Notes

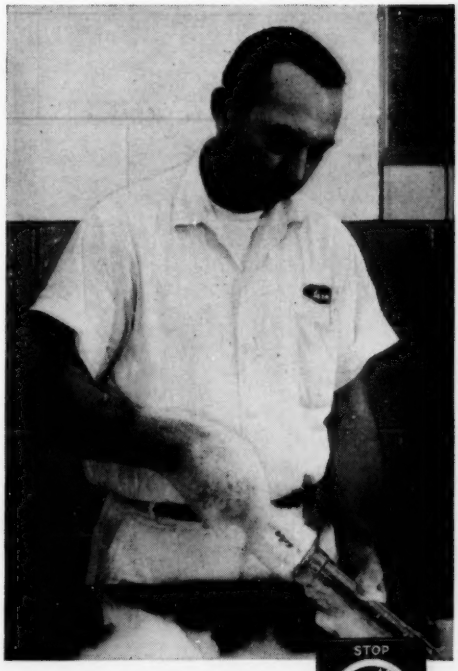
1961 Ohio State Fair

The 1961 Ohio State Fair Dairy Products Competition attracted the largest number of entries in its history. Fifteen block-style and 11 wheel-style Swiss cheeses were judged, as were 18 vanilla ice creams, 17 chocolate ice creams, 15 strawberry ice creams, 15 samples of small curd Cottage cheese, and 12 samples of large curd Cottage cheese.

Professor J. M. Jensen, Michigan State University, discusses Cottage cheese quality at the 1961 Ohio State Fair Cottage cheese clinic.

Supervisor of the contest was DR. T. KRISTOFFERSEN, Associate Professor of Dairy Technology, The Ohio State University. Official judges were R. E. HARDELL, Monroe, Wisconsin, for Swiss cheese, and PROFESSOR J. M. JENSEN, Michigan State University, for ice cream and Cottage cheese. Professor Jensen also conducted clinics in connection with the ice cream and Cottage cheese contests.

The FFA Milk Contest was held for the second year, under the supervision of DR. M. E. GREGORY, Assistant Professor of Dairy Technology, The Ohio State University. Twenty teams, with three members each, participated. Each team member submitted a sample of milk produced under his supervision. Samples were evaluated on the basis of bacteriological and organoleptic quality and composition. In addition, each participant completed a written examination on milk production practices. The contest was won by the Wooster Chapter, coached by GLEN BOLING. The award attached to this placing consists of an expense-paid



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trip to Chicago for the purpose of visiting dairy plants and dairy equipment manufacturers' facilities in that area. Runner-up in the contest was the Shreve Chapter, coached by WILLIAM RUTH.

Dates for two major events sponsored by the Department of Dairy Technology, The Ohio State University, to be held in 1962, were recently announced. The 28th Annual Dairy Technology Conference will begin on February 13 and continue through February 15. The Short Course in Milk Sanitation and Public Health will convene on March 19 and conclude on March 23.

News from Purdue

Dairy Fieldmen's and Dairy Plant Operators' Conferences
November 14, 15

The 1961 Dairy Fieldmen's and Dairy Plant Operators' Conferences are to be held at Purdue University on November 14 and 15, respectively. The conferences are sponsored jointly by the Dairy Department and the Indiana Dairy Products Association.

DR. F. N. ANDREWS, Head of the Dairy Department at Purdue, has announced a Dairy Fieldmen's program which will include papers on The Effect of Mastitis on Milk Composition and Processing Properties, by F. J. BABEL, Dairy Department, Purdue University; Applications of Protein and Fat Determinations as a Basis for Producer Payment, by J. R. BRUNNER, Michigan State University; Increasing Dairy Income by Dairy Beef Production, by T. G. MARTIN, Dairy Department, Purdue University; and Dairying in Brazil, by CARL NOLLER, Dairy Department, Purdue University. The program will also include a panel discussion on the use of dairy field service and a report on a successful low-investment dairy enterprise.

The Dairy Plant Operators' Conference is to include discussions on Automation for Quality Control, by J. R. Brunner, Michigan State University; Modern Concepts of Dairy Plant Management, by C. E. FRENCH, Purdue University, and The Fat-Heart Disease Story, by HUGH MCWILLIAMS, National Dairy Council. The luncheon speaker for the conference will be DON PAARLBERG, former Presidential Economic Adviser, who will discuss some of his experiences as a consultant on national economic policies. The program will also include a discussion on the national quality standards for manufacturing grade milk and dairy plant design for automation.

INDUSTRY NEWS

Two Promotions at Carnation

M. E. ELLERTSON, a graduate of St. Olaf College, and R. W. STACHWICK, a graduate of Montana State College, have been appointed

M. E. Ellertson

R. W. Stachwick

assistant research directors in the Carnation Company Research Laboratories at Van Nuys, California. Both men have had considerable experience in industry and in industrial research.

Borden Company Expands

The Borden Company is expanding its facilities at the Columbus, Ohio, milk plant. The expansion, involving the acquisition of a city block adjacent to the plant, will include the construction of new load-out facilities. These will incorporate the latest in equipment for automatic handling during the loading and unloading process, including case stackers and unstackers, and an extensive conveyor system.

Now in the final stages of construction is a new Borden milk plant in Kettering, Ohio. The company expects to move into the new automated plant in January of 1962.

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DFL Appoints Streblow

Dairyland Food Laboratories, Inc., Waukesha, Wisconsin, announce the appointment of SYLVESTER STREBLOW as sales representative for Northern Wisconsin, Upper Michigan, Minnesota, and North and South Dakota. Streblow has owned and operated Cheese factories in Fond du Lac and Shawano counties for more than 34 yr., and has been responsible for the formation and guidance of many cheese industry associations. He is a former director of the Wisconsin Cheese Makers Association; a former president of the Northeastern Wisconsin Cheesemakers' and Buttermakers' Association; secretary-treasurer of the Tri-County Quality Milk Improvement Association; and president of the Northern Wisconsin Creamery Association.

S. Streblow

MERLE FARNHAM, President of Dairyland Food Laboratories, is touring his company's distant export markets in New Zealand. Purpose of the tour, which may extend as far as Australia, is to secure first-hand knowledge of the rapidly growing export requirements for products.

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Corn Products Company Supports Advanced Studies for Three Foreign Nutritionists

DR. A. L. ELDER, director of Corn Products Institute of Nutrition, has announced that nutritionists from India, Mexico, and the Philippines have been selected as the first recipients of Corn Products Company fellowships at the Columbia University Institute of Nutrition Sciences.

The three students, all experienced in nutrition, will follow an advanced course in public health nutrition designed to develop leaders who can train others and institute programs in their own countries. The Fellows are: DR. RAICHUR K. BHAGAVAN, a nutritionist at the Central Food Technology Institute, Mysore, India; DR. ELIZABETH LOPEZ, a research specialist in nutritional problems of children at the Hospital Infantil de Mexico, Mexico City; and MISS CONCEPCION VELASCO, head of the Nutrition Clinic Laboratory of the Institute of Nutrition of the Philippines, Manila. She has just completed a year's leave of absence during which she performed research on vitaman B_{12} at Johns Hopkins University.

The Columbia University Institute of Nutrition Sciences offers graduate programs in human nutrition and preventive medicine, agriculture and food supply, food technology, and patterns of food habits and how they can be changed.

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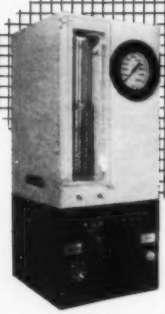

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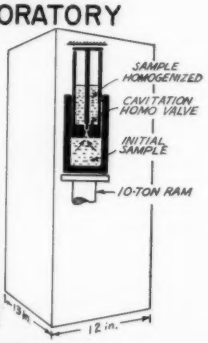

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Economics Laboratory and Klenzade Products Merge

E. B. OSBORN, President of Economics Laboratory, Inc., has announced a merger with Klenzade Products, Inc. of Beloit, Wisconsin. This merger became effective on September 20, with Klenzade continuing as a division of Economics Laboratory, Inc.

Land and Water Use Symposium

An interesting and timely symposium on the use of land and water, with special emphasis on the mountain and plains regions, will be held in the Hilton Hotel in Denver, Colorado, December 27 and 29, 1961. It is sponsored by the American Association for the Advancement of Science, of which the American Dairy Science Association is a cooperating group. DR. R. E. HODGSON, immediate Past-President of A.D.S.A., will be chairman of one session on land and water management.

New Publication from Public Health Service

The publication, Research Grants in Environmental Health in the Public Health Service has been issued by the U. S. Department of Health, Education, and Welfare as PHS Publication No. 870.

The brochure describes briefly many new or enhanced opportunities for research in air pollution, environmental engineering and food protection, occupational health, radiological health, and water supply and pollution control. Areas of primary interest to the Public Health Service in each of the fields are detailed.

Information about other grant programs in environmental health are given in the document, including public health training grants, air pollution traineeship grants, radiological health training grants to institutions, project grants for graduate training in public health (to institutions), regular research fellowships and graduate training grants to institutions.

Copies are available on request from the Office of Resource Development or the Division of Environmental Engineering and Food Protection, Bureau of State Services, Public Health Service, Washington 25, D. C.

Theses Completed

M.S. Degree:

HERBERT L. MILLER—Driver labor cost and related factors in the delivery of ice cream. The Ohio State University.

Ph.D. Degree:

W. R. THOMAS—Enumeration of thermoduric bacteria in milk. Iowa State University.

Dairy Technology Societies

Central Illinois—September 13 meeting was held in Champaign, at the HumKo Company

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Farnum Gray, Southern Dairies, Charlotte, North Carolina
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SUMMARY OF CONTENTS

Section I Objectives and Requirements of the Program

Section II Training Program Schedules for Plant Operation

1. Orientation
2. Plant operations
3. Laboratory
4. Engineering and maintenance
5. Procurement
6. Sales and distribution
7. Office work

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2. Ice cream plants
3. Condensed and dry milk plants
4. Cheese plants
5. Butter plants
6. Evaporated milk plants

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Section III Management and Development

1. Developing skills in personnel management
2. Developing skills in quality control and cost

Many study projects are outlined which are not only essential to management development but may result in savings to the plant that will more than pay the cost of training.

Appendix

1. List of professional and trade organizations
2. List of reference books, booklets, and periodicals

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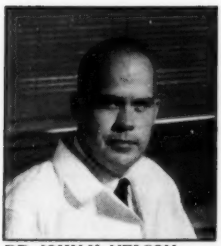
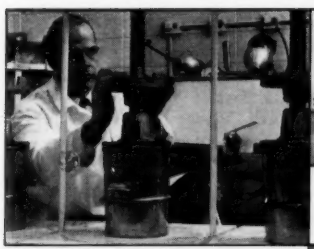


DR. JOHN H. NELSON,
Director of Research

DFL REPORT TO THE CHEESE INDUSTRY NO. 2

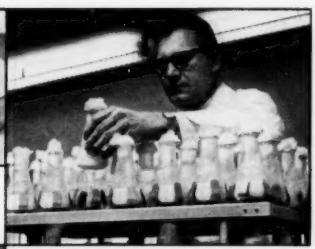
DFL RESEARCH DISCOVERS REMARKABLE CURE AND PREVENTATIVE FOR CALF SCOURS

In this, the second of a series of reports to the Cheese Industry, I would like to show you our Research Department which is continually testing, perfecting, finding new applications for DFL products and searching for entirely new products. Recently, our research led us to the discovery of a highly effective cure and preventative for calf scours. This new scour remedy has successfully passed extensive on-the-farm testing and is now being marketed under the tradename of "PGE." I cordially invite you to visit our Waukesha, Wisconsin plant to see our research department in action and inspect our new, completely automated rennet extract process which is by far the most modern in the world. Until then, here is a brief report on DFL research — its facilities, its people and its purpose.



In this pilot cheese plant, cheese-making trials are conducted to experiment with new product ideas.

Research into product improvements and new products is a vital, essential part of DFL's modern food plant.



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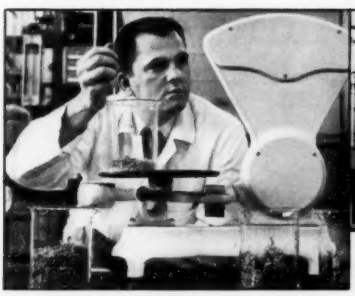

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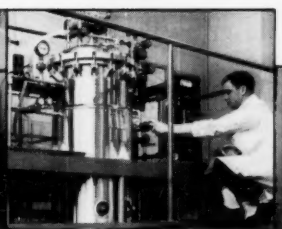
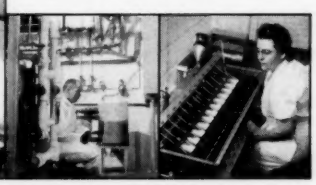


Intricate pilot plant test equipment designed by DFL aids in the search for new products.



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plant. Meeting comprised a tour of the plant plus a talk by Joseph Simon, Professor of Veterinary Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, on Mastitis Control in Dairy Herds in Illinois.

Central Michigan—Fall meeting, September 20, began with a steak dinner, ladies' night meeting, at the High Life Inn. Program Chairman, Larry Curtis, arranged an interesting movie on paper-making by the International Paper Company, for the educational part of the program.

Kansas City—Social hour is scheduled for October 14 at the Ward Parkway Country Club. Dinner and dancing will be featured.

Ohio—Officers of the four Ohio societies are: Cincinnati: V. W. Lein, president; R. Berling, vice-president; R. Deiner, secretary; A. Boerger, treasurer; M. E. Gregory, corresponding secretary. Northeastern Ohio: J. F. Sibert, president; M. Swinehart, vice-president; G. Johnson, secretary; D. Bartholomew, treasurer; N. Berg, sergeant-at-arms. Maumee Valley: E. Grieser, president; P. W. Ling, vice-president; M. George, secretary; R. Heindricks, treasurer; F. Willinger, sergeant-at-arms. Central Ohio: B. Taylor, president; D. Foster, vice president; F. R. Bright, secretary; R. Spires, treasurer; F. March, sergeant-at-arms; T. Kristoffersen, corresponding secretary.

Philadelphia—Speaker at the October 12 meeting was Dr. Theodore A. Kotovitz, Acting Director Research, Chesapeake Instrument Corporation, Shadyside, Maryland; also, Professor of Physics, The Catholic University of America, Washington, D. C. Subject: An Electronic Test for the Measurement of Fat and Solids-not-fat in Milk and Milk Products.

Tri-State—Steubenville Country Club was the locale of the September 20 meeting which featured C. B. Lawrence, Director of Research, G. P. Gundlach & Company, talking on Applying the Physical Effort. Officers for the coming season: Dave Mizer, president; Jim Williams, first vice-president; Herb Fetterolf, second vice-president; Lou Miller, secretary-treasurer; C. J. Honer, corresponding secretary.

Western Michigan—Speaker for the September 18 meeting at Finger's Cafe, Grand Rapids, was Dr. W. K. Moseley of the Moseley Laboratories, Indianapolis, dealing with the milk quality problem.

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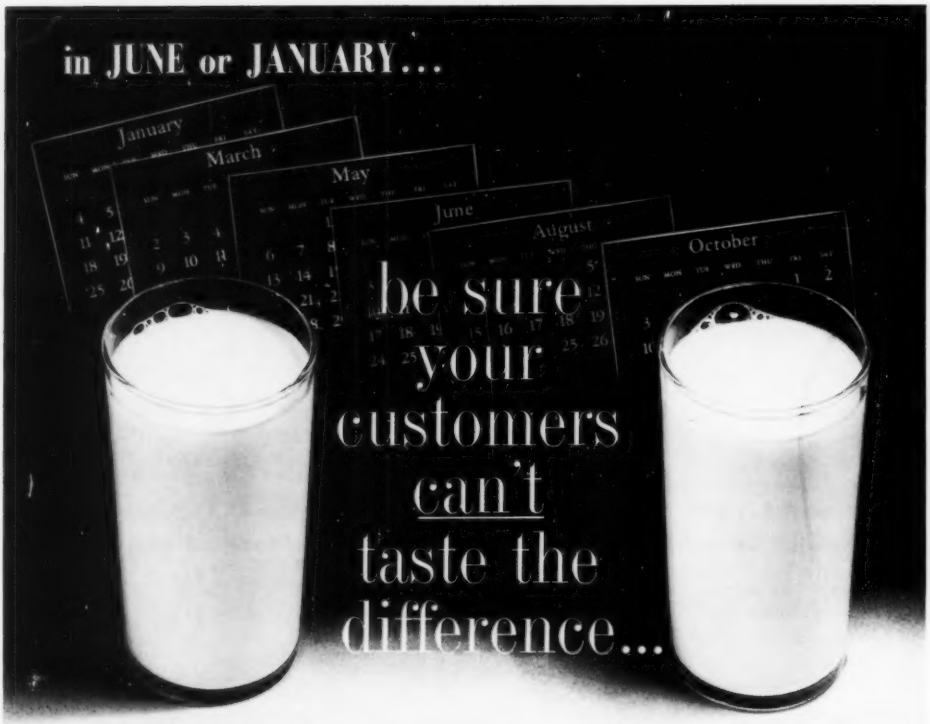

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RESEARCH PAPERS

SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF BITTER WEED FLAVOR IN MILK WITH CHROMOTROPIC ACID

BOOKER T. WHITE[1]
Division of Biochemistry, The Agricultural and Technical College of North Carolina
Greensboro

SUMMARY

A colorimetric method for determining bitter weed flavor in milk has been developed, based upon the reddish-purple color produced in the reaction of the flavor component and/or isotenulin with an acid solution of chromotropic acid. The color produced in the reaction conformed to Beer's Law, and was found to be suitable for the estimation of the flavor component in the concentration range of 0.1 to 1.5 mg/ml. The experimental values for the flavor component were calculated from a standard curve previously prepared for isotenulin at 410 mμ. The factors and experimental conditions which enhanced the accuracy of the method have been described.

The tremendous waste which the dairy industry has encountered from bitter weed contamination (1, 13) in whole milk and dairy products has prompted our efforts to develop a suitable method for its detection and estimation. Preliminary experiments designed to accomplish this end have been guided by the previous works of Buehler (4), Clark (6, 7), and Ungnade (17). The presence of a keto group in tenulin and in its isomer, isotenulin, has been demonstrated in the work reported by Buehler (4) and Clark (6). The latter author prepared through the intermediate compounds, dihydro-isotenulin, $C_{17}H_{24}O_5$, and dihydrotenulin, $C_{17}H_{24}O_5$, their respective phenyl hydrozone derivative, $C_{23}H_{30}O_4N_2$.

Thus, the characteristic keto group which has been shown (6) to be present in both isomeric substances, tenulin and isotenulin, has been observed to respond to an alkaline picric acid reagent to produce a characteristic color reaction. This latter reaction has been employed by Weathers (19), in the development of a method for the estimation of the flavor component in milk.

Recently, we have observed in our laboratory that a characteristic color reaction resulted when either isotenulin or the flavor component from milk was allowed to react with an acid mixture of chromotropic acid. This latter reagent has been employed by Bricker (3) for the estimation of formaldehyde, and more recently by Speck (16) for the estimation of diacetyl.

The method to be described herein for the determination of bitter weed flavor in milk with chromotropic acid is a modification of the procedure and reagents employed by Speck (16) for the estimation of diacetyl.

EXPERIMENTAL PROCEDURE

A. *Solvents.* The solvents used in the preparation of test extracts from bitter weed flavored milk required special methods of purification to free them from

Received for publication December 2, 1960.

[1] Approved by Director of Research as paper No. 1247 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina.

traces of formaldehyde and distillation residues which gave a positive reaction with the test reagent.

1. *Purification of methanol.* Eastman reagent and spectral grades of methanol yielded a distillation residue which interfered with the color reactions for isotenulin and/or the flavor component. This solvent was finally purified by refluxing the solvent with solid sodium hydroxide in the ratio of 10 g. per liter for approximately 4 hr. The treated solvent was distilled and the fraction distilling over at 65° C. was reserved for use in the extraction procedure.

2. *Purification of chloroform.* This solvent was purified with concentrated sulfuric acid according to the procedure recommended by Fieser (9).

3. *Purification of acetone and petroleum ether.* These solvents were purified by procedures recommended by Fieser (9).

B. *Feeding bitter weed meal.* Dried and pulverized leaves and flowering parts of the bitter weed plant were sieved through a 30-mesh screen and the powder packed in No. 10 gelatin capsules. One pound of the powdered material required 33 to 42 capsules.

C. *Production of flavored milk.* The capsules, filled with bitter weed meal, were fed orally with a veterinarian stomach gun in 1-lb. quantities. Samples of flavored milk were taken for analysis at 12-hr. intervals after the first feeding. The data reported in this study covered four different, successive feeding periods.

D. *Isolation of flavor component.*

Step 1. Casein was precipitated from a mixture of 500 ml. of flavored milk, 50 ml. of 85% sulfuric acid, and 1,500 ml. of methanol. After thorough mixing, the casein curd was separated by filtration. The filtrate was successively extracted with 250-ml. aliquots of chloroform. After combining the chloroform extracts, the mixture was treated with excess solid ammonium carbonate, 150 g., to remove excess sulfuric acid, and partially dried with excess sodium sulfate (Extract No. 2).

Step 2. The partially dried chloroform extract (Extract No. 2) was freed of chloroform under vacuum, and the 25 ml. of recovered aqueous concentrate re-extracted with three 50-ml. aliquots of chloroform. After drying the extract (Extract No. 3) with sodium sulfate, the solvent was distilled and the residue digested with 30 ml. of acetone (Extract No. 4). The filtrate from this treatment was concentrated to dryness and the fatty residue extracted with 25 ml. of distilled water. Fatty material was removed from the aqueous extract (Extract No. 5) by shaking with two 50-ml. volumes of petroleum ether. The combined lower layers from the ether treatment were freed of organic solvents and diluted to 25 ml. with distilled water. This aqueous extract (Extract No. 6) was reserved for estimation of the flavor component.

E. Estimation procedure.

1. *Reagents.*

(a) Sulfuric acid solution—10 ml. of water were diluted to 100 ml. with concentrated sulfuric acid.

(b) Chromotropic acid solution—A sample of 2.5 g. of Eastman reagent grade chromotropic acid was diluted to 100 ml. with water. The reagent was stable for five days. It was found advisable to store the unused reagent in a brown bottle at ice box temperature.

2. *Development of reddish-purple color.*

(a) One milliliter of the test solution (Extract No. 6) was introduced into a 150-mm. test tube and the tube surrounded with ice water.

(b) To the test solution was added 1 ml. of 2.5% chromotropic acid solution. This was followed with the addition of 4 ml. of the (9 : 1) sulfuric acid solution.

(c) After careful mixing of the contents of the test tube, the tube was transferred to a boiling water bath and heated at 100° C. for exactly 15 min. The tube was removed immediately and cooled under running water.

(d) For estimation, 3 ml. of the colored mixture were introduced into a 1-cm. cell and the absorption determined at 410 mμ with the Beckman Model DU Spectrophotometer against a reagent blank similarly prepared.

For routine measurements, the Klett-Summerson Photoelectric colorimeter was adequate. With this instrument Filter No. 42 was employed.

DISCUSSION AND RESULTS

Factors affecting the chromotropic acid reaction.

1. Effects of sulfuric acid concentration:

The data reported by Speck (16) for diacetyl estimation served as a reference in evaluating the acid concentration appropriate for maximum color development. It was noted that an acid concentration below 80% produced very little color when the reaction was carried out according to the procedure recommended by Speck. When concentrated sulfuric acid was employed, the test solution darkened. This darkening of the solution indicated destruction of the isotenulin molecules. Maximum color development was secured with a 90% sulfuric acid concentration.

2. Effect of chromotropic acid concentration.

The color development for given concentrations of isotenulin and 90% sulfuric acid was noted to vary linearly up to a concentration of 5% chromotropic acid. For concentrations of isotenulin up to 1.5 mg., 2.5% chromotropic acid was found to be adequate.

3. Effect of time of development.

When the reaction was carried out with 2.5% chromotropic acid and 90% sulfuric acid, color development was observed to increase slowly after the first 5-min. interval. Full color development occurred within 15 min. when the reaction was carried out in a boiling water bath at 100° C.

Our preliminary experiments revealed that isotenulin responded to most of the reagents commonly employed to characterize carbohydrates. The various reagents studied in this connection and the results obtained have been tabulated in Table 1. It will be observed that positive reactions were obtained with chromotropic acid (3, 16), catechol (12), picric acid (2), and triphenyltetrazolium

TABLE 1
Color responses of isotenulin and lactose

Reagent	Isotenulin	Lactose
Anthrone	(8)−[a]	+
Catechol	(12)+	+
Chromotropic Acid	(3, 16)+	+
DI (OH) Naphthalene	(5)−	+
Fehling's	(10)−	+
O-Phenylene Diamine	(20)−	+
Phospho-Molybdic Acid	(11)−	+
Picric Acid	(2)+	+
T-Phenyl-T-Zolium Chloride	(15)+	+

Minus (−), negative response.
Plus (+), positive response.
[a] Literature references.

chloride (15), whereas the remainder gave negative responses. As isotenulin did not respond to the anthrone sulfuric acid test, this reagent served to guide us in preparing lactose-free test extracts. The positive responses noted for isotenulin, with the various test reagents listed in Table 1, were also positive for the flavor constituent in concentrated solutions. In similar manner, each positive test reagent gave a negative response with control extracts prepared from nonflavored milk.

Our observation of the chromotropic acid reaction with isotenulin would suggest the cleavage-formation of formaldehyde or some similar constituent during the course of the reaction. This latter observation was further verified through a modification of the procedure recommended by Verman (18) for the detection of various hydrocarbons. Verman observed that an acid formaldehyde reagent produced characteristic color reactions for various hydrocarbons. In our modification of Verman's procedure, a color was produced when isotenulin and a sulfuric acid mixture of paraphenylphenol in chloroform were allowed to react at elevated temperature. This reaction would indicate that formaldehyde or some similar substance was formed during the reaction and that the color formation resulted from a condensation of the cleavage product with para-phenylphenol in an acid medium.

TABLE 2
Concentration of bitter weed flavor estimated by chromotropic acid reagent

Sample	Hours after feeding	Pounds consumed	Conc. mg/ml	Mg. conc. ½ liter flavored milk
1	Nonflavored milk	None	0.00	0.00
2	12	1	0.206[a]	5.150[b]
3	12	1	0.803	20.075
4	12	1	1.034	25.850

[a] Estimations were made upon 1-ml. aliquots of 25 ml. of extract No. 6 obtained from ½ liter of flavored milk.
[b] The values listed give the total concentration in 25 ml. of extract No. 6 for ½ liter of flavored milk.
Numbers 2, 3, 4 represent consecutive feeding periods.

TABLE 3

Recovery of flavor in duplicate determinations and added isotenulin

Sample	Conc. mg/ml	Conc. mg/½ liter flavored milk
4 (1)	1.034 mg/1 ml.	25.85/½ liter
4 (2)	1.01010 mg/ml	25.25
4 (3)	0.514—Detn.	00.514—Thr/25 ml.
4 (4)	1.200 mg/ml (found)	1.264 mg/25 ml. (theoretical)

Samples 4 (1, 2) represent duplicates for ½ liter of flavored milk processed.

Sample 4 (3)—25-ml. test extract No. 6; 4 (1, 2) was diluted to 50 ml. for estimation.

Sample 4 (4)—25 ml. of 4 (3) was diluted with 0.750 mg. of isotenulin and concentration estimated.

It has been noted that the estimation procedures reported for formaldehyde (3) and diacetyl (16) determinations depicted absorption curves with maximum absorption occurring at 570 mμ. Other analytical procedures which yielded formaldehyde under various conditions (14) indicated maximum absorption for these reactions also at 570 mμ. In contrast with these reports, the data depicted in Figure 1 for chromotropic acid and isotenulin indicated maximum absorption for this, reacting at 410 mμ.

This report by Bricker (3) included acrolein as an interference in the estimation procedure for formaldehyde. Glyceride interference was only slightly discernible in Step No. 1 of the present procedure with chromotropic acid. This slight defect was eliminated in Step No. 2 by extracting the aqueous extract (Extract No. 5) with petroleum ether prior to treatment with the color-producing reagent.

The interference often encountered from solvent contamination was readily detected by varying the sulfuric acid concentration in the test procedure with chromotropic acid. With a sulfuric acid concentration of 76%, only solvent contamination gave a positive response with the test reagent. When a sulfuric acid concentration below 80% was used in preparing the test reagent, neither the flavor component nor isotenulin was observed to yield a color response.

Our data for the application of the chromotropic acid reagent for the estimation of bitter weed flavor in milk have been presented in Tables 2 and 3. From the standard curve, Figure 2, a value of 0.570 was calculated for K from the following equation:

$$K \text{ equals } D/C$$

where D is the optical density and C the concentration of isotenulin per milliliter of test solution. The concentration of the flavor component in 1 ml. of test extract (Extract No. 6) was calculated by substituting in the above formula.

It was noted that test extracts containing isotenulin, in a concentration of 40 μg. per milliliter in 50 ml. of extract, did not register an objectionable, bitter flavor. When the concentration was raised to 50 μg. per milliliter for 50 ml. of test extract, the bitter flavor was definitely detectable. For the estimations depicted in Table 2, aliquots of 100-500 ml. of flavored milk were used in the preparation of the 25-ml. aqueous test extracts (Extract No. 6). In

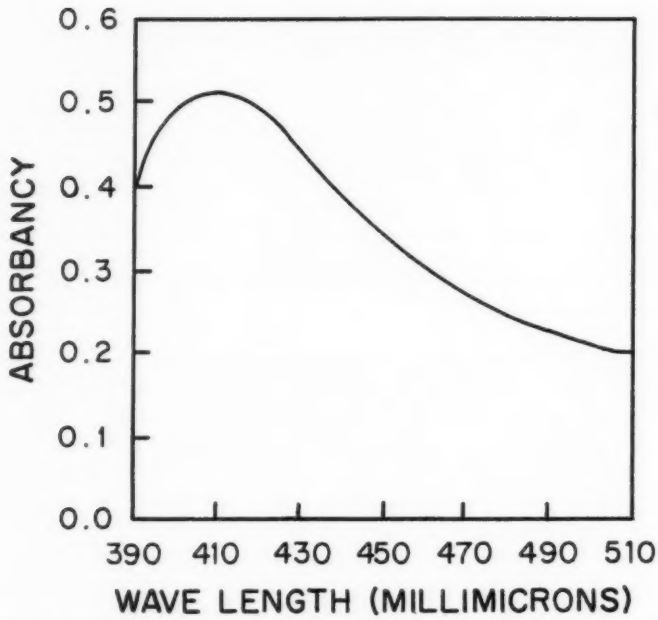

FIG. 1. Absorption spectrum chromotropic acid and isotenulin M.P. 158° C. Sample concentration 0.9420 mg. per milliliter.

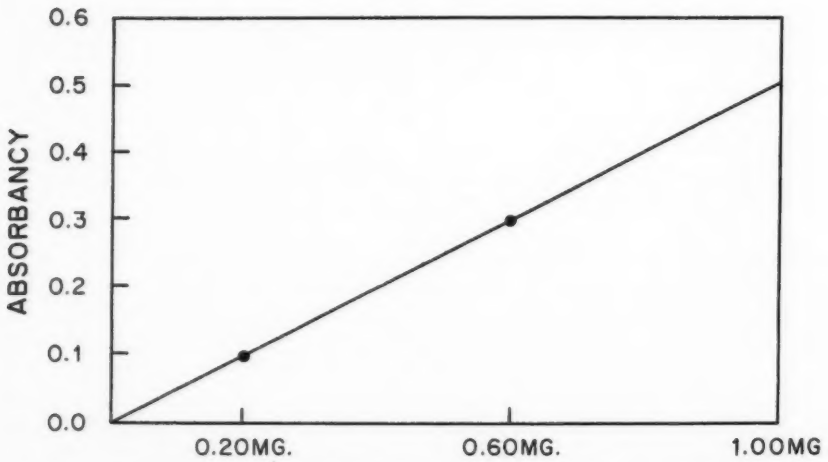

FIG. 2. Concentration of isotenulin (milligrams of isotenulin per milliliter) at 410 mμ.

general, the concentration of the flavor component per milliliter of the 25-ml. test extract (Extract No. 6), prepared from 500 ml. of flavored milk, was 100 μg. per milliliter.

In this concentration range our chromotropic acid reagent could be used with either the Model DU Spectrophotometer or the Klett-Summerson photoelectric colorimeter, for the detection and estimation of bitter weed flavor in milk.

It was noted, during the three-day feeding period reported in this communication, that the concentration of the flavor component increased after the first, second, and third feeding periods of 1-lb. samples. The concentration of the flavor component obtainable for a 1-lb. sample reached a maximum on the fourth day. This observation seems valid for duplicate analyses depicted in Table 3, conducted upon several ½-liter aliquots of the flavored milk collected for the fourth feeding period.

In addition, the data in Table 3 would also confirm the accuracy of our estimation procedure for the flavor component. When isotenulin was added to a diluted test extract (Extract No. 6), a fairly quantitative increment was noted in optical density.

Thus, the data presented in Tables 1, 2, and 3 would seem to verify the accuracy of our procedure for the estimation of bitter weed flavor in milk.

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PHOSPHATE HEAT TREATMENT OF MILK TO PREVENT BACTERIOPHAGE PROLIFERATION IN LACTIC CULTURES

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SUMMARY

The effects of various prosphate salts on bacteriophage proliferation in milk cultures and the binding of calcium in milk were studied, with the aim of developing a practical and reliable method for preventing bacteriophage development in lactic starter cultures. The kind and concentration of phosphate, pH, and heat treatment had a marked effect on phage inhibition, and the free calcium content of milk. Of the group of phosphate salts tested individually in milk, orthophosphates showed the greatest degree of bacteriophage inhibition. Most of the phage types tested were suppressed by 2% orthophosphate (salt), but the most resistant types required 3%. Heating the milk after adding the phosphate was essential to sufficiently bind most of the free calcium. Usually, the free calcium content of the treated milk ranged from 10 to 30 p.p.m. The phosphated milk became more effective as the pH of the phosphate buffer and milk was increased from pH 6.4 to 7.0. The best combination for phage inhibition, minimum milk precipitation, and economy was obtained when milk was heated with 1.7% orthophosphate salt, pH 6.6, followed by the addition of 0.3% pyrophosphate. Thirteen different lactic strains grossly contaminated with their respective phages were freed of phage within three to four subcultures in phosphated milk. In most instances, the activity of the cultures in phosphated milk was as great as or greater than in the controls.

The requirement of divalent metals, especially calcium, for the proliferation of bacteriophage (phage), has been established (1, 4, 8). Particular attention has been given to the calcium requirements of lactic phages, because of the economic importance of starter failures in making cheese and other cultured dairy products (4, 9). Several attempts have been made to develop a medium which would prevent or suppress the proliferation of phage in lactic cultures. Potter and Nelson (10) developed a calcium-deficient, semichemically defined medium. Doull and Meanwell (5) proposed a sodium citrate–containing medium. More recently, Reiter (11) developed a calcium-deficient milk medium designated as a phage resistant medium, or simply as P.R.M.

Because of an apparent inhibitory effect of P.R.M. on some strains of lactic bacteria (3), and the lack of a suitable milk medium for propagating phage-free starters, this laboratory has investigated the feasibility of adding phosphate to lactic starter-milk with the aim of chemically binding the essential calcium and thereby preventing phage proliferation in lactic cultures.

Preliminary results (2, 7) showed that the addition of 2% orthophosphate to milk, combined with a mild heat treatment, prevented the development of almost all types of lactic phages. Since then, the effects of kind and concentration of phosphate, pH, heat treatment, and percentage of milk solids on the

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binding of calcium, the inhibition and elimination of phage proliferation, and the activity of lactic cultures have been determined in greater detail. Typical data from the accumulated results are summarized here.

MATERIALS AND GENERAL METHODS

Bacteria and phages. Thirty-four strains of lactic streptococci and homologous phages were either isolated from cheese and cultured buttermilk or obtained from other laboratories.

Stock suspensions of phages were prepared from lysed homologous lactic strains grown in sterilized skimmilk. The curd was precipitated with 85% lactic acid and removed by filtering. The whey was neutralized and passed through a Seitz ST-1 filter.

Phage activity. The phage-host relationship of each of the 34 lactic strains was determined by spotting serial dilutions of each phage on agar plates seeded with an individual bacterial strain. Thirteen different types of phage were indicated and each type was employed in the study.

Quantitative phage determinations were made by a two-layer agar plaque technique. In addition to plaque counts, the phage titers were frequently determined with the dilution end point method in sterile skimmilk.

In most instances, the effectiveness of a particular phosphate treatment was determined by inoculating the milk wth fixed volumes (1%) of culture and homologous phage and measurng the phage titers after each of several passages in the phosphated milk.

Phosphate solutions. For most of the work, concentrated stock solutions (50% w/v) orthophosphate buffer, pH 6.55, were prepared by dissolving 30 g. of KH_2PO_4 and 20 g. Na_2HPO_4 in distilled water with the aid of heat and bringing the volume to 100 ml. An appropriate volume of stock solution was added to milk to yield the desired concentration of phosphate (w/v). A buffer concentrate (50% w/v) containing equal amounts of K_2HPO_4 and $NaH_2PO_4 \cdot H_2O$, pH 6.63, was added to milk to obtain a 3% concentration. Varied ratios of primary and secondary phosphates were used in preparing concentrated solutions (50-60% w/v) having different pH levels. Concentrated stock solutions of pyrophosphate contained (50% w/v) tetrapotassium pyrophosphate.

Calcium analysis. The indirect titration method of Yalman *et al.* (12) was used in determining the ionizable calcium content of the milk. Analyses were made on the phosphate-treated and nontreated skimmilks, and on their supernatants following centrifugation for 90 min. at 40,000 r.p.m. A 25-ml. sample was evaporated to dryness on a steam bath, the solids determined, and then reduced to ash overnight in a muffle furnace at 650° C. The calcium values for the supernatants are reported as unbound calcium in parts per million.

Bacterial activity. The activity of lactic bacteria following growth in phosphate-containing milk was determined by a 6-hr. activity test. Inoculation was at the rate of 1% and incubation was at 88° F. The effect of the phosphate milk on the growth of *Leuconostoc* strains was determined by plate count.

The number of *Leuconostoc* cells in mixed lactic cultures was determined with a selective plating medium developed in this laboratory (to be published).

EXPERIMENTAL PROCEDURES AND RESULTS

Effect of phosphate concentration. Stock (50% w/v) orthophosphate buffer, pH 6.55, was added to fresh skimmilk to yield 1.0, 1.5, 2.0, and 3% of phosphate by weight. The phosphated milks and a control were heated at 100° C. for 15 min., cooled, and individual tubes inoculated with 1% of *Streptococcus cremoris*, Strains R-1, C-13, and ML-1, and their homologous phages. The phage titer in each culture was determined at inoculation, after incubation for 18 hr. at 72° F., and after each of three subcultures in the respective phosphated milk.

The data in Table 1 show that the phage for C-13 was suppressed by 1% phosphate, R-1 by 1.5 to 2%, and ML-1 by 3.0%. Other results showed that 2% phosphate was sufficient to prevent the proliferation of most types of lactic phage. However, two types, both specific for Lactic Strains C-1 and C-10, required as much as 3%.

TABLE 1

Effect of orthophosphate concentration on phage proliferation

Phosphate	Unbound calcium	Lactic strain	Phage titer (No/ml)				
			Inoculum	18-hr. culture	Subculture 1st	Subculture 2nd	Subculture 3rd
(%)	*(p.p.m.)*						
None	228	R-1	22×10^6	92×10^7	*	*	*
1.0	66	R-1	22×10^6	76×10^7	*	*	*
1.5	35	R-1	22×10^6	91×10^4	12×10^4	20×10^4	810
2.0	30	R-1	22×10^6	115×10^4	48×10^2	20	0
2.5	24	R-1	22×10^6	180×10^4	8×10^3	80	0
3.0	16	R-1	22×10^6	145×10^4	8×10^3	10	0
None	228	C-13	11×10^6	132×10^7	*	*	*
1.0	66	C-13	11×10^6	1×10^5	4×10^3	12	0
1.5	35	C-13	11×10^6	13×10^4	630	0	0
2.0	30	C-13	11×10^6	23×10^4	140	0	0
2.5	24	C-13	11×10^6	34×10^4	100	0	0
3.0	16	C-13	11×10^6	63×10^4	380	0	0
None	228	ML-1	48×10^5	136×10^7	*	*	*
1.0	66	ML-1	48×10^5	24×10^8	*	*	*
1.5	35	ML-1	48×10^5	20×10^8	*	*	*
2.0	30	ML-1	48×10^5	31×10^8	*	*	*
2.5	24	ML-1	48×10^5	57×10^4	30×10^5	18×10^5	12×10^6
3.0	16	ML-1	48×10^5	8×10^3	20×10^2	40	0

* Culture lysed, no subculture.

The content of free calcium in phosphated milk decreased greatly with increased concentration of phosphate.

Effect of pH. Orthophosphate buffers having pH values in the range of 6.25 to 7.4 were added at a 2% by weight level to fresh skimmilk and the treated milks were heated at 100° C. for 15 min. The resulting milks, representing pH values from 6.3 to 7.1, were used in testing phage strains for *S. cremoris* KH and ML-1. The phage titers at the time of inoculations and after each of three subcultures in the respective media are shown in Table 2. The data clearly show

TABLE 2

Effect of pH of phosphated milk on phage proliferation

pH of buffer	pH of milk	Calcium content	Lactic strain	Phage titer (No/ml)				
				Inoculum	18-hr. culture	Subculture 1st	Subculture 2nd	Subculture 3rd
		(p.p.m.)						
6.25	6.3	44	KH	12×10^5	17×10^8	*	*	*
6.32	6.35	34	KH	12×10^5	46×10^8	*	*	*
6.4	6.42	33	KH	12×10^5	20×10^3	128×10^2	5×10^3	0
6.55	6.5	24	KH	12×10^5	21×10^3	15×10^3	0	0
6.88	6.68	21	KH	12×10^5	49×10^2	100	0	0
7.00	6.76	19	KH	178×10^5	11×10^3	20	0	0
7.10	6.88	20	KH	178×10^5	9×10^3	50	0	0
7.2	7.0	16	KH	178×10^5	2×10^3	30	0	0
7.4	7.1	13	KH	178×10^5	3×10^3	30	0	0
6.55	6.5	24	ML-1	26×10^4	58×10^7	$>10^7$	*	*
6.88	6.68	21	ML-1	26×10^4	36×10^3	84×10^6	*	*
7.00	6.76	19	ML-1	26×10^4	17×10^3	41×10^3	63×10^4	27×10^3
7.10	6.88	20	ML-1	26×10^4	23×10^3	24×10^2	49×10^2	110
7.2	7.0	16	ML-1	26×10^4	118×10^2	280	0	0
7.4	7.1	13	ML-1	26×10^4	94×10^2	30	0	0

* Culture lysed, no subculture.

a marked influence of pH on phage inhibition and elimination, the higher pH levels being much more effective. A pH of 6.4 to 6.55 in the milk was required to free Strain KH. Tests with other phage types showed that pH 6.55 was sufficient to eliminate most phage strains from the lactic cultures. However, with 2% phosphate, a pH of a least 7.0 was required to inhibit phage for Strains ML-1 and C-10. The calcium levels in the treated milks decreased slightly as the pH of the buffer was increased.

Effect of heating. Sterile stock buffer, pH 6.55, was added aseptically to flasks of sterile (251° F.—15 min.) and pasteurized (143° F.—30 min.) skimmilk to provide a 2% level of phosphate salt. Individual flasks of pasteurized phosphated milks were heated at 100° C. for 15, 30, 60, and 90 min., respectively. Individual flasks of sterile phosphated milks were heated for 15 min. at temperatures ranging from 70 to 100° C.

Another series of pasteurized and sterile milks was treated with pH 7.28 buffer at the 2% level and given similar heat treatments. Milks with pH 6.55 buffer were inoculated with Lactic Strain R-1 and its phage, and milks with pH 7.28 buffer were inoculated with Strain ML-1 and its phage. The effects of heating phosphate-containing milks are shown in Table 3. A minimum heat treatment of 100° C. for 30 min. was required when pasteurized milk was the starting medium, but much less heat was needed with sterile milk. A temperature of 80° C. for 15 min. was sufficient with R-1 phage; however, 95 to 100° C. was required with other strains. Heating had a marked effect on the chemical binding of the calcium by the phosphate. Increases in temperature and period of exposure usually resulted in lesser amounts of free calcium in the milks.

Effect of milk solids. Stock buffer, pH 6.55, was added at the 2% phosphate level to raw and sterile skimmilks and to raw and sterile skimmilks freshly reconstituted from NFDM powder to levels of 8, 10, 12, and 14% solids.

TABLE 3

Effect of heating phosphated milk on phage proliferation

Type of milk	Heat treatment	Calcium content	Lactic strain	Phage titer (No/ml)				
				Inoculum	18-hr. culture	Subculture 1st	Subculture 2nd	Subculture 3rd
	(° C.)	(p.p.m.)						
Sterile skim, no phosphate	None	270	R-1	22×10^6	6×10^8	*	*	*
Sterile skim, 2% phosphate pH 6.55	None	157	R-1	22×10^6	19×10^7	*	*	*
Sterile skim, 2% phosphate pH 6.55	70–15 min.	56	R-1	22×10^6	25×10^6	43×10^5	28×10^6	*
Sterile skim, 2% phosphate pH 6.55	80–15 min.	58	R-1	22×10^6	18×10^4	1×10^4	120	0
Sterile skim, 2% phosphate pH 6.55	85–15 min.	48	R-1	22×10^6	138×10^4	21×10^4	110	0
Sterile skim, 2% phosphate pH 6.55	90–15 min.	27	R-1	22×10^6	105×10^4	6×10^3	40	0
Sterile skim, 2% phosphate pH 6.55	95–15 min.	25	R-1	22×10^6	95×10^4	2×10^4	20	0
Sterile skim, 2% phosphate pH 6.55	100–15 min.	21	R-1	22×10^6	9×10^5	7×10^3	50	0
Pasteurized skim, 2% phosphate pH 6.55	None	169	R-1	22×10^6	66×10^7	*	*	*
Pasteurized skim, 2% phosphate pH 6.55	100–15 min.	44	R-1	22×10^6	11×10^5	1×10^4	14×10^3	56×10^3
Pasteurized skim, 2% phosphate pH 6.55	100–30 min.	22	R-1	22×10^6	115×10^4	48×10^2	20	0
Pasteurized skim, 2% phosphate pH 6.55	100–60 min.	18	R-1	22×10^6	125×10^4	6×10^3	60	0
Pasteurized skim, 2% phosphate pH 6.55	100–90 min.	24	R-1	22×10^6	12×10^5	7×10^4	30	0

TABLE 3 (contd.)

Type of milk	Heat treatment	Calcium content	Lactic strain	Phage titer (No/ml)				
				Inoculum	18-hr. culture	Subculture 1st	Subculture 2nd	Subculture 3rd
	(° C.)	(p.p.m.)						
Pasteurized skim, 2% phosphate pH 7.28	None	157	ML-1	24×10^5	1×10^7	*	*	*
Pasteurized skim, 2% phosphate pH 7.28	100–15 min.	27	ML-1	24×10^5	21×10^4	14×10^4	37×10^3	56×10^4
Pasteurized skim, 2% phosphate pH 7.28	100–30 min.	18	ML-1	24×10^5	58×10^3	15×10^2	20	0
Pasteurized skim, 2% phosphate pH 7.28	100–60 min.	14	ML-1	24×10^5	31×10^3	6×10^2	30	0

* Culture lysed, no subculture.

The sterile and fresh milks were steamed for 15 and 30 min., respectively. Each milk was tested with Lactic Strain R-1 and its phage. Table 4 lists the

TABLE 4

Effect of milk solids on 2% phosphate treatment

Type of milk	Phosphated milk	Calcium content	R-1 phage titer (No/ml)			
			Inoculum	18-hr. culture	Subculture 1st	Subculture 2nd
	(pH)	(p.p.m.)				
Fresh skim	6.52	35	22×10^6	77×10^4	1×10^4	20
Fresh reconstituted						
8% NFDM	6.60	10	22×10^6	61×10^4	16×10^3	0
10% NFDM	6.55	24	22×10^6	9×10^5	6×10^3	30
12% NFDM	6.52	29	22×10^6	20×10^5	15×10^3	70
14% NFDM	6.48	30	22×10^6	5×10^5	21×10^4	95×10^2
Sterile skim	6.36	31	22×10^6	86×10^4	6×10^3	50
Sterile reconstituted						
8% NFDM	6.42	15	22×10^6	83×10^4	48×10^2	20
10% NFDM	6.44	26	22×10^6	8×10^5	2×10^4	80
12% NFDM	6.40	37	22×10^6	25×10^5	18×10^5	28×10^3
14% NFDM	6.40	35	22×10^6	112×10^4	71×10^2	60

phage inoculum and the phage titers after one growth period and two subcultures in the respective media. The data with Strain R-1 indicate that the solids content of milk treated with phosphate was not too significant. However, tests with other lactic strains indicated that the phosphate treatment was more effective with low solids milk.

Effect of pyrophosphate. In an attempt to increase the effectiveness of the orthophosphate buffer in milk, varied quantities of pyrophosphate were tested in combination with orthophosphate. Reconstituted NFDM (10%) was divided into five lots and stock orthophosphate concentrate, pH 6.55, was added to yield 2.0, 1.9, 1.8, 1.7, and 1.6%, respectively. All lots were heated at 100° C. for 30 min. Then tetrapotassium pyrophosphate (50% concentrate) was added to yield 0.1, 0.2, 0.3, and 0.4% by weight in the lots of hot milk which already contained 1.9, 1.8, 1.7, and 1.6% orthophosphate, respectively. The fifth lot containing 2% orthophosphate served as a control.

The milks were cooled immediately and inoculated with *S. cremoris* ML-1 and its resistant phage. The phage titers at inoculation and after each of four subcultures in the respective media are presented in Table 5. All levels of pyro-

TABLE 5

Effect of pyrophosphate as a supplement in orthophosphated milk

Potassium pyrophosphate	Orthophosphate buffer	Calcium content	ML-1 phage titer (No/ml)				
			Inoculum	18-hr. culture	Subculture		
					1st	2nd	3rd
(%)	*(%)*	*(p.p.m.)*					
None	2	24	450,000	80×10^6	32×10^7	*	*
0.1	1.9	20	450,000	12×10^4	14×10^4	175×10^3	30×10^5
0.2	1.8	13	450,000	47×10^3	15×10^3	7×10^3	200
0.3	1.7	17	450,000	80×10^3	4×10^3	0	0
0.4	1.6	19	450,000	13×10^3	0	0	0

* Culture lysed, no subculture.

phosphate in combination with orthophosphate were more effective in suppressing bacteriophage proliferation than the 2% level of orthophosphate alone. Pyrophosphate at the 0.4% level had a marked reducing effect on the phage titer; no phage was detected after the first subculture.

Comparison of phosphate-treated milks. Thirteen strains of lactic bacteria, each having a different phage sensitivity pattern, and their homologous phages were tested in milks containing 2 and 3% orthophosphate buffer pH 6.63 and in milks containing 1.7% orthophosphate supplemented with 0.3% potassium pyrophosphate, to determine the number of transfers required to free each of phage. Three series of flasks containing pasteurized skimmilk forewarmed to 130° F. were treated with sufficient stock orthophosphate buffer pH 6.63 to yield 1.7, 2, and 3% by weight of the phosphate salt.

All flasks were heated at 100° C. for 30 min. Then, while still hot, the 1.7% orthophosphate was supplemented with 0.3% tetrapotassium pyrophosphate. All milks were cooled and one flask from each of the three phosphate treatments was inoculated with one of the lactic strains and its phage. Phage titers were determined after inoculation and after each of four serial subcultures in the same medium. They are presented in Table 6. All but two of the 13 different lactic strains were freed of phage with 2% orthophosphate. Results obtained with 3% orthophosphate were similar, except that the two resistant phages

TABLE 6

Effect of orthophosphate on different lactic phages

Lactic strain	Phage titer (No/ml)					
	Inoculum	18-hr. culture	Subculture			
			1st	2nd	3rd	4th
2% orthophosphate, pH 6.55						
R-1	25×10^6	49×10^4	32×10^2	10	0	0
US-3	1×10^8	28×10^4	9×10^2	10	0	0
ML-1	46×10^4	16×10^4	20×10^5	89×10^7	*	*
KH	23×10^6	17×10^4	160	10	0	0
HP	27×10^6	22×10^4	30	10	0	0
K	41×10^6	65×10^5	38×10^3	8×10^2	20	0
E-8	68×10^6	50×10^4	16×10^2	400	10	0
C-2	12×10^6	98×10^3	26×10^2	10	0	0
C-3	90×10^6	15×10^6	20×10^3	100	10	0
C-10	52×10^4	30×10^6	51×10^7	*	*	*
C-13	62×10^6	42×10^5	11×10^2	10	0	0
H-1	29×10^6	18×10^4	6×10^3	200	10	0
H-4	86×10^4	43×10^3	6×10^2	10	0	0
3% orthophosphate, pH 6.63						
ML-1	15×10^4	33×10^3	45×10^2	20	0	0
C-10	2×10^5	6×10^4	15×10^2	0	0	0

* Culture lysed, no subculture.

were eliminated from the lactic cultures. All 13 lactic types were freed of phage within two or three subcultures, in milk containing 1.7% orthophosphate supplemented with 0.3% pyrophosphate (Table 7).

TABLE 7

Effect of mixture of 1.7% orthophosphate and 0.3% pyrophosphate on different lactic phages

Lactic strain	Phage titer (No/ml)				
	Inoculum	18-hr. culture	Subculture		
			1st	2nd	3rd
R-1	125×10^5	58×10^3	21×10^2	0	0
US-1	20×10^6	40×10^3	20	0	0
ML-1	52×10^4	18×10^3	100	0	0
KH	90×10^5	27×10^4	60×10^3	50	0
HP	54×10^5	25×10^4	200	0	0
K	45×10^6	11×10^5	60×10^2	70	0
E-8	60×10^5	50×10^2	10	0	0
C-2	40×10^5	59×10^3	300	0	0
C-3	43×10^5	90×10^3	120	0	0
C-10	32×10^4	23×10^4	30×10^3	130	0
C-13	45×10^5	40×10^4	900	0	0
H-1	29×10^6	12×10^5	88×10^2	20	0
H-4	77×10^5	52×10^3	60	0	0

Effect of orthophosphate on activity of lactic cultures. The activities of single and mixed lactic cultures were determined after growth in skimmilks containing 2 and 3% orthophosphate.

Sterile, concentrated sodium and potassium phosphate buffers were prepared by mixing the phosphate salts in the ratios listed in Table 8, to yield final pH

TABLE 8

Effect of different orthophosphate salts on culture activity

Buffer combination				Ratio	Phosphate	pH	Titratable acidity (ml. N/20 NaOH) Culture			
No.	Monobasic		Dibasic				W1	W2	W3	W4
					(%)					
....	None	—	.....	.....	....	.....	10.4	11.3	12.1	11.5
1	$NaH_2PO_4 \cdot H_2O$	—	Na_2HPO_4	3–1	2.0	6.24	11.1	12.4	13.4	12.2
2	$NaH_2PO_4 \cdot H_2O$	—	Na_2HPO_4	2–1	2.0	6.52	9.9	9.8	10.4	9.9
3	$NaH_2PO_4 \cdot H_2O$	—	Na_2HPO_4	1–1	2.0	6.8	7.0	8.2	6.8	7.1
4	$NaH_2PO_4 \cdot H_2O$	—	K_2HPO_4	1–1	2.0	6.62	12.8	12.4	13.0	14.6
5	$NaH_2PO_4 \cdot H_2O$	—	K_2HPO_4	1–1	3.0	6.62	10.2	12.1	12.9	12.0
6	$NaH_2PO_4 \cdot H_2O$	—	K_2HPO_4	1–2	2.0	7.0	12.0	12.4	11.8	12.3
7	$NaH_2PO_4 \cdot H_2O$	—	K_2HPO_4	1–4	2.0	7.28	11.1	12.8	11.0	13.4
8	KH_2PO_4	—	Na_2HPO_4	3–2	2.0	6.57	11.0	11.1	12.4	12.6
9	KH_2PO_4	—	Na_2HPO_4	3–2	3.0	6.57	8.9	11.4	10.5	9.0
10	KH_2PO_4	—	Na_2HPO_4	1–1	2.0	6.82	8.8	10.6	10.1	8.2
11	KH_2PO_4	—	K_2HPO_4	1–1	2.0	6.8	13.4	12.8	12.9	12.7
12	KH_2PO_4	—	K_2HPO_4	1–1	3.0	6.8	10.7	11.5	12.7	12.0

* pH of 2% aqueous phosphate buffer.

values ranging from 6.24 to 7.28. The buffer solutions were added to sterile skimmilks in sufficient amounts to yield 2 and 3% of each salt. The phosphated milks were heated at 100° C. for 15 min., cooled, and inoculated with lactic culture. In most instances the lactic cultures were carried for two subcultures in the phosphated milk prior to determining their activity. Inoculation was at the rate of 1% and incubation was at 72° F. for 18 hr. The titratable activities of four representative commercial mixed lactic starters following growth in phosphated milks are listed in Table 8. Similar results were obtained with single strains.

The activity of several lactic cultures was reduced after they had been propagated for two transfers in phosphated milk containing only the sodium salt. The reduced activity was more striking as the pH of the buffer (No. 2, 3) was increased. However, the potassium salts (No. 11, 12), regardless of pH or concentration (2 to 3%), had no inhibitory effect on lactic cultures. Potassium and sodium phosphate buffer combinations (No. 4, 5, 6, 7, 8) had no inhibitory effect when equal ratios of sodium and potassium ions were present.

Effect of combination of ortho- and pyrophosphate on activity of lactic cultures. Single lactic strains and mixed commercial cultures were tested for acid production after at least two subcultures in milks containing varied percentages of both ortho- and pyrophosphate. Sufficient concentrated orthophosphate buffer, pH 6.63, was added to six lots of sterile skimmilk to yield 3, 2, 1.9, 1.8, 1.7, and 1.6% phosphate. Each lot of phosphated milk was steamed for 15 min. The portions with 2 and 3% orthophosphate received no further treatment. Pyrophosphate concentrate (50%) was added to the remaining four lots of milk in sufficient amounts to bring the total phosphate salt content to 2%. The reaction of the treated milks was approximately pH 6.5. The titratable acidities of four representative commercial mixed lactic cultures are presented in Table 9. In other studies, slight inhibition occurred with a few single strains

TABLE 9

Effect of tetrapotassium pyrophosphate on culture activity

Tetrapotassium pyrophosphate	Orthophosphate pH 6.6	Titratable acidity (ml. N/20 NaOH)			
		Culture			
		W1	W2	W3	W4
(%)	(%)				
None	None	13.2	12.5	12.8	10.1
None	2	15.0	13.1	14.7	14.5
None	3	10.0	12.3	11.0	10.4
0.1	1.9	12.7	12.6	12.7	12.9
0.2	1.8	10.3	12.3	12.5	10.1
0.3	1.7	9.8	9.6	12.7	11.8
0.4	1.6	9.0	11.5	12.6	12.4

when 0.4% pyrophosphate was added as a supplement; however, most single strains, like most mixed strains, remained as active as the controls without phosphate. To avoid slight inhibition of culture activity with some strains, 0.3% pyrophosphate should be used in combination with 1.7% orthophosphate.

DISCUSSION

In screening tests, various types of phosphates, including orthophosphate, metaphosphates, tripolyphosphates, and pyrophosphates were added to milk to determine their effect on phage proliferation. Orthophosphates were definitely more effective than any of the others and had less inhibitory effect on lactic culture activity. Pyrophosphates and tripolyphosphates tested individually were ineffective against phage, but they definitely improved the effectiveness of the orthophosphate treatment. However, they were only effective when added in the proper sequence, which was as follows: heating the milk, adding orthophosphate, steaming the mixture, and adding pyrophosphate or polyphosphate to the hot milk. Recently, Galesloot (6) showed that the lysis of a nisin-producing strain could be prevented by 1% polyphosphate alone. In our studies, polyphosphates alone had very little effect on most phage types. The simple addition of concentrated orthophosphate buffer, pH 6.6, to yield 2% phosphate by weight, followed by heating the milk at 100° C. for 30 to 45 min., was sufficient to prevent proliferation of most phage types and 3% prevented all types. However, 2% was sufficient to inhibit all types when the milk was buffered to pH 7.0.

Most of the orthophosphate concentrates containing different salt combinations could be added to milk in sufficient amount to provide 2% by weight of the salt without causing casein precipitation. However, some combinations did cause precipitation at the 3% level (for example, K_3PO_4: $NaH_2PO_4 \cdot H_2O$—ratio 3:7, pH 6.55).

All phosphate salts had to be added to the milk in the form of a liquid concentrate to prevent casein precipitation. Also, forewarming the milk to 130 to 150° F. and heating it immediately after the addition of phosphate was an essential procedure. Phosphate concentrates could be added aseptically to sterile

milk without precipitation, provided the milk had been previously autoclaved at 120° C. for 15 min.

The addition of pyrophosphates to milks previously treated with orthophosphates did not appear to markedly reduce the content of unbound calcium, although its addition actively suppressed bacteriophage development. Therefore, it seems evident that the pyrophosphate plays another role in preventing phage proliferation in milk, probably by complexing other divalent metals.

In most instances, the activity of mixed- and single-strain lactic cultures grown in phosphated milk was equal to or greater than that of control cultures. No significant difference was found between the *Leuconostoc* content of mixed lactic cultures grown for four to six successive transfers in phosphated milk and that in the controls. Incubation periods less than 18 hr. at 72° F. did reduce the *Leuconostoc* content.

Kay (8) observed actual destruction of *Salmonella typhi* phage when exposed to solutions of phosphate buffer, citrate, and versene. In the present study, no loss in phage titers was observed when bacterial free phage filtrates were stored in phosphate-treated milks for 1 wk. Results of phage adsorption studies indicated that the phosphate added to milk greatly impaired adsorption of the phage to the host cell.

Results of this study offer two means for preventing the proliferation of phage in lactic bacterial starters, as follows:

1. To fresh or reconstituted skimmilk (9-14% solids), forewarmed to 130° F., add sufficient concentrated sodium-potassium orthophosphate (50%) buffer (Na_2HPO_4 and KH_2PO_4—Ratio 2:3), pH 6.5-6.6, to yield 1.7% of phosphate salt by weight, and steam the treated milk 30 to 45 min. While it is still hot, add sufficient concentrated solution (50%) of tetrapotassium pyrophosphate to yield 0.3% by weight of this salt. Cool and inoculate the phosphated milk in the usual manner and incubate for 18-20 hr. at 72-77° F.

2. To fresh or reconstituted skimmilk (9-12% solids) forewarmed to 130° F., add sufficient concentrated (50%) buffer (equal amount of K_2HPO_4 and $NaH_2PO_4 \cdot H_2O$, pH 6.6) to yield 3% phosphate salts by weight and steam the treated milk 30 to 45 min. Cool and inoculate the phosphated milk in the usual manner. Incubate for 18-20 hr. at 72-77° F.

The first procedure is preferred, because it is slightly more effective against phage proliferation, is less apt to cause milk precipitation, and reagents are more economical.

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IDENTIFICATION OF SUBSTANCES IN MILK CULTURES OF *PSEUDOMONAS FLUORESCENS* WHICH STIMULATE LACTIC STARTER CULTURES [1]

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SUMMARY

Milk cultures of a strain of *P. fluorescens* incubated ten days at 30° C. were fractionated to identify the factor(s) which stimulated acid production by lactic starter cultures. Eight series of fractionations were made and the fractions tested on four lactic cultures. The fraction precipitated by acetone and ethanol increased titratable acidity an average of 28% over the control at 14 hr. of incubation at 21° C. This fraction also produced early coagulation of lactic starter cultures. Autoclaving the precipitate destroyed both the stimulatory activity and the early coagulation effect. Dialysis only slightly reduced the activity of the precipitate fraction. It appeared that enzyme activity was involved in stimulation, but this could not be clearly established. The amino acid fraction, which was stimulatory, contained 17 amino acids and gave an average increase in titratable acidity of 13%. Preliminary attempts to further purify the stimulatory factor from the precipitate fraction were unsuccessful.

Stimulation of acid production in milk through associated growth of lactic acid–producing organisms and other bacterial species has been noted by various investigators (1, 3, 5, 6). The increased acid production generally has been attributed to protein degradation products made available to the acid-producing bacteria by the other organisms. Hall (4) observed that a cell-free filtrate of *Bacterium coli* added to milk stimulated acid production by lactic starter cultures. Noting that addition of the filtrate some time prior to inoculation with lactic culture increased the stimulatory effect, she concluded that enzyme action was a factor. Gyllenberg *et al.* (3) have recently reported that initial growth of pseudomonads in milk accelerated the multiplication of acidogenic bacteria.

Stimulation of lactic starter cultures by cell-free filtrates from milk cultures of *Pseudomonas fluorescens* was reported by Claydon and Koburger (2). The stimulation appeared to be due partly to protein fractions and partly to enzyme action of the filtrate. The study reported here was undertaken in an effort to identify the factor(s) responsible for this stimulation.

METHODS

The general procedure was to measure the titratable acidity produced by lactic starter cultures containing the various fractions obtained from milk cultures of the *P. fluorescens* organism.

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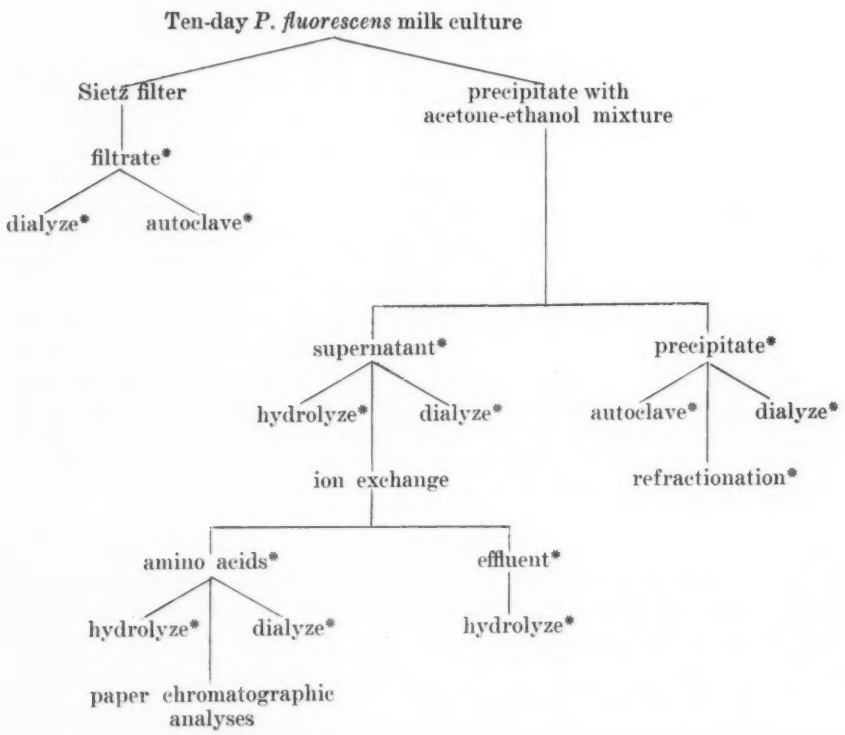
Propagation and culture of P. fluorescens. The strain of *P. fluorescens* previously referred to (2) was propagated in litmus milk with weekly transfer. Following initial development at 21° C., cultures were held refrigerated until the succeeding transfers. To prepare the culture fractions, 100-ml. quantities of reconstituted nonfat milk solids (made to 9% solids in distilled water) were dispensed in 250-ml. Erlenmeyer flasks and autoclaved for 20 min. at 15-lb. pressure. After cooling, the milk was inoculated with *P. fluorescens* and incubated at 30° C. for ten days, with daily shaking. Obvious proteolysis occurred in one day and progressed with holding. To determine if the nature of the medium had an effect on production of stimulatory material, *P. fluorescens* culture was also grown in synthetic medium, from which fractions were obtained for testing. The medium contained sodium acetate (0.5%), secondary potassium phosphate (0.1%), magnesium sulfate (0.1%), and ammonium sulfate (0.1%).

Preparation of culture fractions. The scheme of fractionation is given in Figure 1. A portion of the ten-day milk culture was removed and Sietz-filtered.



* Tested for stimulatory ability.

FIG. 1. Schematic diagram of culture fractionation.

This filtrate was used as a reference to determine the response of the starter cultures to the addition of the stimulatory agents. Both the filtrate and subsequent fractions were stored at −5° C. until tested.

To 40 ml. of culture was added 160 ml. of a 3 to 1 mixture of acetone and ethanol to precipitate proteins, enzymes, and cells. The mixture was held at −5° C. for 4 hr., then centrifuged at 800 G. for 15 min. The clear supernatant was decanted, reduced in volume under vacuum to near dryness, then readjusted to the original volume (40 ml.) with sterile distilled water. This clear solution was free of precipitable material when treated with 15% trichloroacetic acid. The precipitate portion was placed under vacuum in a 40° C. water bath to remove the solvent. It was then brought to the original 40-ml. volume with sterile distilled water.

Fractionation of supernatant. Free amino acids were removed from the supernatant fraction in an ion exchange column (Amberlite IR-120) in the hydrogen cycle. Twenty milliliters of the supernatant was diluted with four volumes of ethanol and applied to the top of the column. Effluents were checked for complete removal of amino acids by the ninhydrin reaction. The columns were washed with distilled water until a negative Molisch test for lactose was obtained. The amino acids adsorbed on the column were eluted with a 2% ammonia solution. Both the effluent and the eluate were reduced in volume under vacuum to near dryness, then readjusted to original volume with sterile distilled water.

Preparation of hydrolyzed fractions. Portions of the supernatant, amino acids, and effluent fractions were hydrolyzed to break down any complex nitrogenous material that might have been present. One volume of the material to be hydrolyzed was added to 3 vol. of 5 *N* HCl and autoclaved for 7 hr. at 15-lb. pressure. After hydrolysis, samples were repeatedly dried and wetted with water on a steam bath, to remove excess acid. These samples were then adjusted to pH 6.5 with 0.1 *N* NaOH and brought to final volume.

Preparation of dialyzed fractions. To remove low molecular weight materials from the various fractions, they were dialyzed against distilled water at 5° C. for three days with six changes of water. (Dialyzer tubing 4465-A2, A. H. Thomas Company.)

Refractionation of precipitate. In attempts to separate the stimulatory agent(s) from the precipitate fraction, resuspended precipitate was dialyzed as above, then fractionated by gradient addition of acetone and/or ammonium sulfate. The ammonium sulfate containing fractions were dialyzed against distilled water for three days and the acetone fractions were placed under vacuum to remove the solvent, then made back to volume.

Paper chromatographic analysis for amino acids. The eluate from the ion exchange column was assayed for free amino acids by two-dimensional descending paper chromatography. Butanol–acetic acid–water (5:1:4 by vol.) was the first-dimension solvent. Phenol saturated with water in an atmosphere of ammonia and hydrogen cyanide gas was used as the second-dimension solvent. Amino acids were identified after location with ninhydrin by comparison of Rf values and relative locations with chromatograms of known amino acids. Tryp-

tophane was identified by its reaction with Erlich's reagent following one-dimensional descending paper chromatography.

Lactic starter cultures. Mixed strain commercial cultures obtained from supply laboratories were used in testing the various fractions for stimulation. These were propagated in autoclaved, reconstituted nonfat milk solids (previously tested to insure freedom from inhibitory substances) made to 9% solids. These cultures were transferred weekly, incubated at 21° C., and held at refrigerated temperatures between transfers. In trials investigating stimulatory effects, freshly transferred cultures in initial stages of coagulation were used as inoculum.

Testing of prepared fractions. As a basis of comparing the stimulatory effect of various fractions, all fractions prepared were adjusted with distilled water to a volume equal to the original amount of the *P. fluorescens* culture used for fractionating. To measure acid production, reconstituted milk (9% solids) was dispensed in 100-ml. quantities in screw-cap bottles and autoclaved for 20 min. at 15-lb. pressure. After adjusting to 21° C., each 100-ml. lot was inoculated with 1% lactic starter culture, then with the fraction being tested. These fractions, prepared as previously described, were added to the milk at the rate of 1%, since with cell-free filtrates this amount usually gave optimum stimulation. Equal-volume adjustments were made on the controls with sterile distilled water. After the prepared samples were mixed, 9-ml. quantities from each 100-ml. lot were dispensed into six sterile screw-cap tubes. This provided duplicate tubes of the same sample for testing at three different incubation periods. Tubes were incubated at 21° C. and titratable acidities determined at 10, 12, and 14 hr. by titrating with 0.1 *N* NaOH. Results were reported as the mean of two titrations, which seldom differed more than $\pm$ 0.02% acid. Eight series of fractionations of milk cultures of the *P. fluorescens* organism were made and the fractions obtained were tested on four lactic starter cultures. Two separate trials were made with each culture.

RESULTS

Where stimulation was obtained, it was greater at 14 hr. incubation than at 10 and 12 hr. (Table 1). Therefore, description of results is limited to those obtained at the 14-hr. period.

Effect of filtrate. Addition of 1% cell-free filtrate to the lactic starter cultures resulted in an average increase in titratable acidity over the control samples of 36% at 14 hr. The samples containing this cell-free filtrate also coagulated at lower acidities than the control samples (i.e., approximately 0.4% for test samples as compared with approximately 0.7% for controls). The filtrate was proteolytic on milk agar plates and the proteolytic enzyme(s) may account for the early coagulation.

Dialyzing the filtrate resulted in a decrease in titratable acidity as compared with the nondialyzed filtrate. The dialyzed filtrate produced an average increase of 23% acidity over the control samples at 14 hr., and early coagulation of the milk also occurred. Autoclaving the filtrate for 15 min. at 15-lb. pressure reduced stimulation much more than did dialysis and gave only a 13% increase in

TABLE 1

Summary of effects of various fractions of the *P. fluorescens* milk cultures on acid development by lactic starter cultures

Fraction [a]	Incubation at 21° C.		
	10 hr.	12 hr.	14 hr.
	Average titratable acidity in per cent [b]		
Control	0.35	0.43	0.53
Filtrate	0.42	0.56	0.72***
Autoclaved filtrate	0.37	0.45	0.60***
Dialyzed filtrate	0.37	0.49	0.65***
Precipitate fraction	0.40	0.50	0.68***
Autoclaved precipitate	0.36	0.44	0.54ns
Dialyzed precipitate	0.38	0.48	0.64***
Supernatant	0.36	0.44	0.59***
Hydrolyzed supernatant	0.37	0.44	0.59***
Dialyzed supernatant	0.34	0.40	0.51ns
Amino acid fraction	0.38	0.48	0.60***
Hydrolyzed amino acids	0.38	0.47	0.58***
Dialyzed amino acids	0.35	0.43	0.52ns
Effluent	0.35	0.43	0.53ns
Hydrolyzed effluent	0.34	0.42	0.52ns
Precipitate + amino acids + effluent	0.41	0.52	0.69***

[a] Fractions were added to 100-ml. lots of milk and contained the equivalent amount of material present in 1 ml. of the *P. fluorescens* milk culture.

[b] Averages of results of eight trials (two with each of four lactic cultures).

*** Significantly different from the controls at the 0.001 level at 14 hr.

ns Not statistically different from the controls at 14 hr.

acidity over the control samples at 14 hr. No early coagulation of the milk was observed with the autoclaved filtrate.

Effect of precipitate fraction. Addition of the precipitate fraction to lactic starters stimulated acid production, increasing the titratable acidity an average of 28% over that of the controls at 14 hr., and coagulation of the milk also occurred at low acidities. The precipitate was proteolytic on milk agar plates. Autoclaving the precipitate destroyed its stimulatory effect as well as its coagulating ability, suggesting that enzymes probably were involved. Dialyzing the precipitate reduced its stimulatory effect only slightly. This reduction may have been due to loss during dialysis of low molecular weight materials that were occluded in the precipitate during its formation. Early coagulation of the milk also occurred with this fraction.

Effect of supernatant fraction. Adding the supernatant fraction to milk resulted in an average increase of 11% in titratable acidity over the controls at 14 hr. The supernatant was not proteolytic on milk agar plates nor did it contribute to early coagulation of the milk. Dialysis of the supernatant removed the stimulatory material from this fraction. Hydrolyzing the supernatant failed to alter its stimulatory effect as compared with the nonhydrolyzed supernatant, indicating that the stimulatory agents in this fraction were probably amino acids.

Effect of amino acid fraction. The amino acid fraction obtained from the ion-exchange column, when added to milk, was stimulatory. At 14 hr. there was an average increase of 13% titratable acidity over the control samples. Hydrolyzing the amino acid fraction had little effect on its stimulatory nature,

whereas dialyzing the amino acid fraction completely removed the stimulatory material. Chromatographic analyses of the amino acid fraction showed that it contained 17 amino acids. These were: aspartic, glutamic, serine, glycine, threonine, alanine, tyrosine, valine, phenylalanine, leucine/isoleucine, proline, histidine, lysine, arginine, methionine, and tryptophane. An unknown substance suggesting cysteine was present, but it could not be positively identified.

Effect of effluent fraction. The effluent fraction had no stimulatory effect, indicating that the stimulatory nature of the *P. fluorescens* culture lay with the free amino acids and the precipitate fraction. Hydrolyzing the effluent fraction had no effect on its activity.

When the precipitate fraction was recombined with the amino acid fraction and the effluent fraction (in proportions present in the original culture), the stimulation obtained was almost equal to that produced by the filtrate. The difference could arise from losses during preparation of fractions.

Preliminary attempts to isolate the stimulatory substance(s) from the precipitate fraction were unsuccessful. Refractionation by gradient addition of ammonium sulfate and/or acetone resulted in no distinct separation of stimulatory from nonstimulatory fractions.

In testing fractions of the *P. fluorescens* cultures grown in synthetic medium, no stimulation was obtained from the filtrate, the solvent-precipitate, or the supernatant (data not shown). These fractions were not proteolytic on milk agar plates nor did they cause early coagulation of the milk.

DISCUSSION

Results of the study indicate that the stimulation obtained from the *P. fluorescens* culture arose from the free amino acids and the precipitate fraction, with the latter providing the greater stimulation. The exact nature of the stimulatory factor in the precipitate is still obscure.

Since autoclaving the precipitate completely destroyed its stimulatory nature, it appears that an enzyme(s) or other heat labile factor was involved. If the growth stimulants were wholly nonenzymatic, it is doubtful that autoclaving would so drastically alter them that they would become completely inactive. On the other hand, the stimulatory factor must be relatively heat stable, since it was previously reported (2) that placing the filtrate in a boiling water bath for 5 min. only slightly reduced its activity.

The early coagulation of lactic starter cultures containing added precipitate or filtrate was probably due to a proteolytic enzyme(s). However, it seems doubtful that this enzyme was involved in stimulation, since it had been previously shown (2) that adding various amounts of filtrate to the milk substrate prior to inoculation with lactic starters gave no greater response than when filtrate and inoculum were added concurrently.

Although it is generally recognized that peptides contribute to lactic culture stimulation, in this study the stimulatory nature of the *P. fluorescens* culture arose from amino acids and larger fractions of the precipitate portion. Evidently, a complex substrate such as milk is necessary for the production of

stimulatory substances, since none was obtained when *P. fluorescens* was grown in the synthetic medium.

ACKNOWLEDGMENT

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EFFECT OF DEAERATION ON THE FLAVOR STABILITY OF CONCENTRATED SWEETENED CREAM

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SUMMARY

Adequate deaeration markedly lessened flavor deterioration in concentrated sweetened cream during storage for 6 mo. at 40 and 70° F. The effectiveness of deaeration increased as the temperature at which it was initiated was increased from 135 to 170° F. and as the period of deaeration was lengthened from 0 to 60 min. On the other hand, addition of oxygen to concentrated sweetened cream accelerated the rate of flavor deterioration. The peroxide values of deaerated samples decreased slightly during storage, whereas those of nondeaerated samples increased greatly.

Concentrated sweetened cream (1, 6) contains approximately 40% fat. As with other milk products, the development of off-flavors such as stale, oxidized, and rancid, must be guarded against. A sucrose content of 60-65% in the water portion furnishes the necessary protection from quality-damaging bacteria when high-quality cream is used and adequate sanitation is maintained.

The purpose of this work was to investigate the efficacy of removing oxygen by deaeration in maintaining the flavor of this product.

The equipment used for preparing the samples was pilot-plant scale.

Preparation of concentrated sweetened cream. Whole milk was pasteurized at 170° F. for 15 sec. or at 150° F. for 30 min., separated, and the cream standardized to 65% fat. The cream was warmed to 130-140° F. and sucrose and nonfat dry milk (NFDM) were added at the rate of 52 and 10 lb., respectively, per 100 lb. of 65% cream. Thus, each 162 lb. of the final product, concentrated sweetened cream, contained approximately: 65 lb., or 40% of milk fat; 52 lb., or 32% of sucrose (62% in the sucrose-water portion); 13 lb. or 8% of nonfat milk solids (3 lb. from the cream and 10 lb. added); and 32 lb., or 20% of water.

This blend of heavy cream, sucrose, and NFDM was heated in some experiments (Figures 1, 2, and 3) for 15 sec. at 170° F. and tempered immediately as required in a continuous (tubular) operation, before it was deaerated as described below; in others (Figure 4 and 5) it was heated to 150° F., deaerated, and then given a final heat treatment at 150° F. for 30 min. in small hermetically sealed cans to insure the prevention of growth of molds (4) and other undesirable organisms.

Deaeration. Batches of concentrated sweetened cream were divided into two parts. One part was the control, the other was deaerated, except as noted, by flashing it into an unheated vacuum pan at a temperature within the range of 135 to 170° F. with the vacuum at approximately 29 in. of mercury, holding it momentarily, breaking the vacuum, and sampling and then holding under the vacuum for 30 min., again sampling and again deaerating for 30 min. At the

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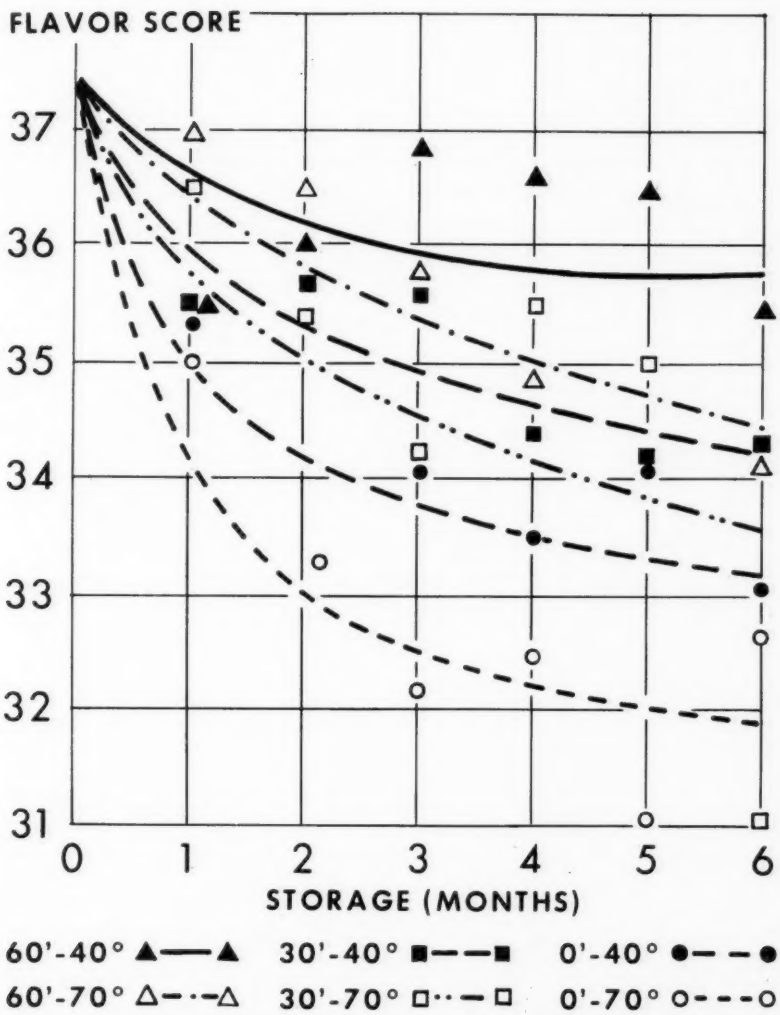

FIG. 1. Effect of deaerating and vacuum-packing concentrated sweetened cream on its flavor score during storage at 40 and 70° F., when the temperature of the concentrate pumped to the unheated vacuum pan was 135° F. and the holding time was 0, 30, and 60 min.

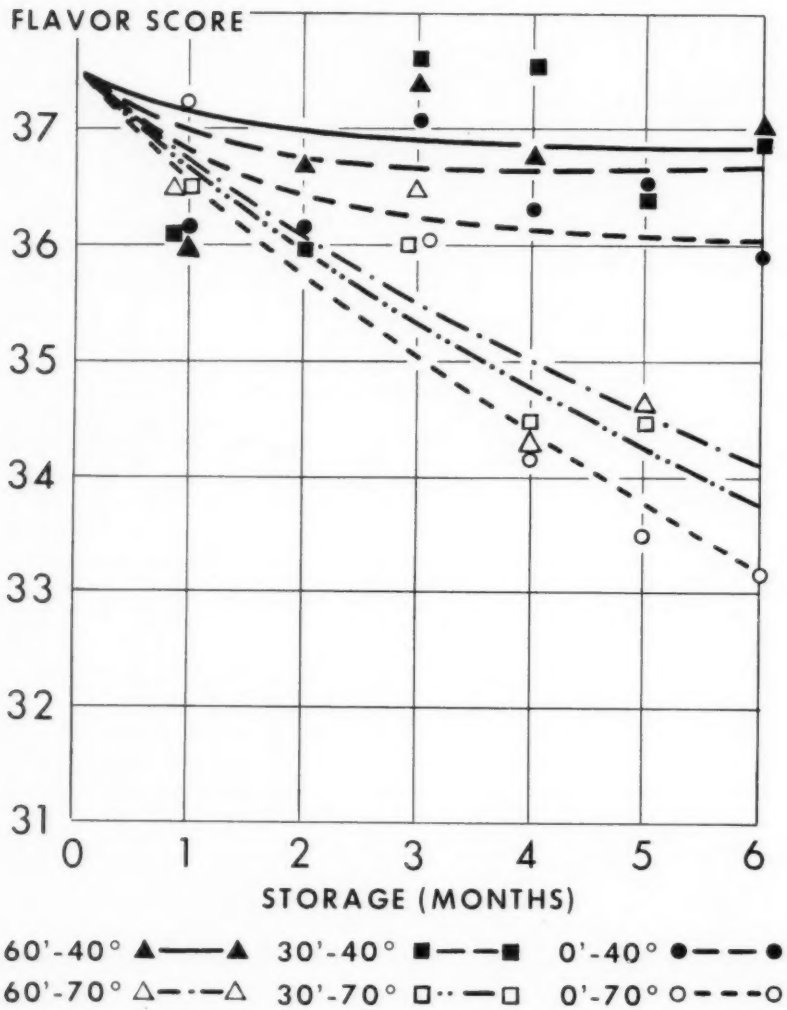

FIG. 2. Effect of deaerating and vacuum-packing concentrated sweetened cream on its flavor score during storage at 40 and 70° F., when the temperature of the concentrate pumped to the unheated vacuum pan was 150° F. and the holding time was 0, 30, and 60 min.

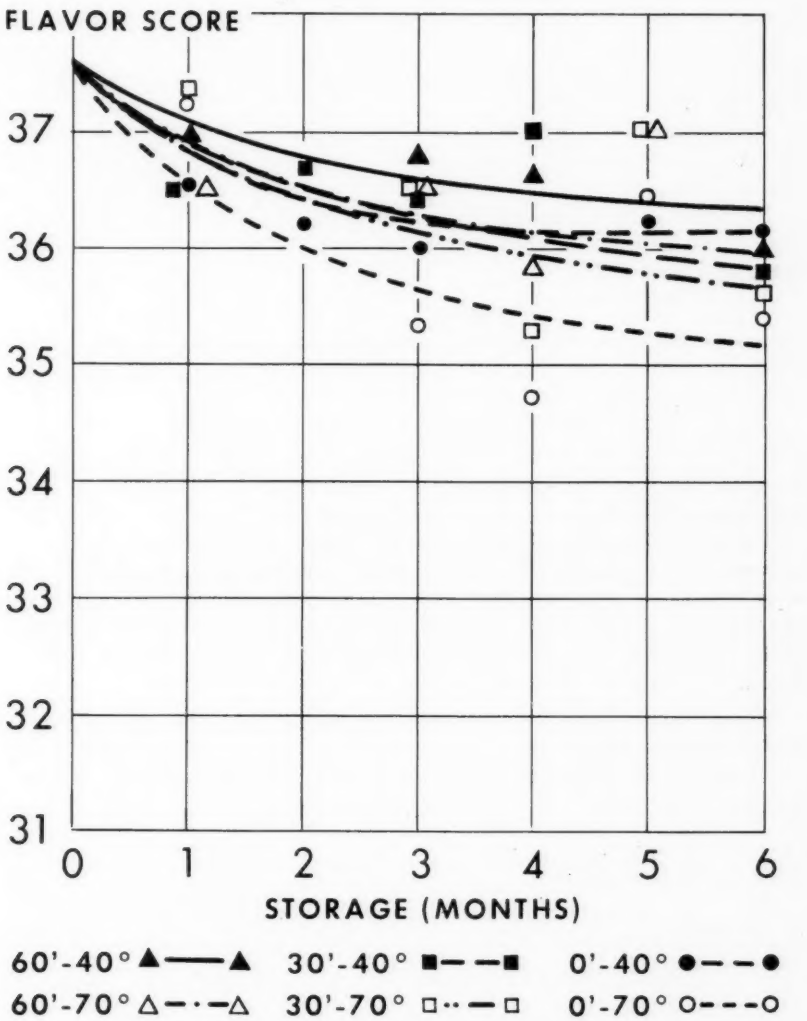

FIG. 3. Effect of deaerating and vacuum-packing concentrated sweetened cream on its flavor score during storage at 40 and 70° F., when the temperature of the concentrate pumped to the unheated vacuum pan was 170° F. and the holding time was 0, 30, and 60 min.

end of each of these three deaerating periods, the product was allowed to flow at atmospheric pressure into small cans to within ⅛-in. of the top. Air in the head space of the cans was removed in a sealer under 25 in. of vacuum. During deaeration, the water content of the concentrated sweetened cream was reduced 1 to 3%.

Control samples were cans of the nondeaerated product that were filled and sealed at atmospheric pressure.

Oxygenation. In two experiments, after sucrose and NFDM were added to the cream in the proper proportions, the blend was divided into two parts. One part was used for preparing control samples as described above. Into the other portion at 105-110° F., oxygen was bubbled for 1 hr. Cans were filled with this product, sealed under oxygen, and held 30 min. at 150° F. before being placed in storage at 40 and 70° F.

ANALYSIS

On opening a can, the entire content was mixed thoroughly to obtain uniform distribution of solids, and of the surface portion where flavor deterioration was most likely to occur.

Flavor scoring. For tasting, the concentrated sweetened cream was diluted with an equal volume of distilled water to reduce both its sweetness and viscosity. If the product did not disperse satisfactorily in the water, the mixture was hand-homogenized.

The taste panel consisted of six trained judges, each in an isolated booth. It used the scoring guide illustrated in Table 1, and each member independently recorded his score. The averages of these scores are reported here to the nearest month of tasting. The scoring guide is based partly on the score-card and scoring guide of the American Dairy Science Association for dry milk, but includes flavors not listed on that score-card.

Peroxide analysis. Three methods of recovering fat from the concentrated sweetened cream were tested. Separation by holding samples overnight at 0° F., warming and centrifuging a portion of each was unsatisfactory because of

TABLE 1

Scoring guide for concentrated sweetened cream

Flavor	Slight	Definite	Pronounced
Cooked	40-39	38-37	36-35
Caramel	39-38	37-36	35-34
Scorched	36-34	33-31	30-28
Stale	36-34	33-31	30-28
Utensil	36-34	33-31	30-28
Oily	35-34	33-32	31-30
Proteolytic	35-33	32-30	29-27
Oxidized	35-33	32-30	29-27
Metallic	35-33	32-30	29-27
Musty	34-32	31-29	28-26
Yeasty	34-32	31-29	28-26
Tallowy	32-30	29-26	25-23
Rancid	32-30	29-26	25-23
Fishy	30-28	27-24	23-21
Foreign	25-20	19-10	9-0

variation in the amount of fat obtained. The detergent method of Stine *et al.* (5) gave values impossible to interpret. The procedure described by Pont (3) was found to be satisfactory and was employed for extracting the fat used in determining peroxide values by the method of Hills and Thiel (2). Peroxide values are reported in milliequivalents of oxygen per kilogram of fat. Benzoyl peroxide added to the solvent was 98.1% recovered.

RESULTS AND DISCUSSION

Effect of deaerating conditions. The conditions under which air was removed from the concentrated sweetened cream affected its flavor stability. This is

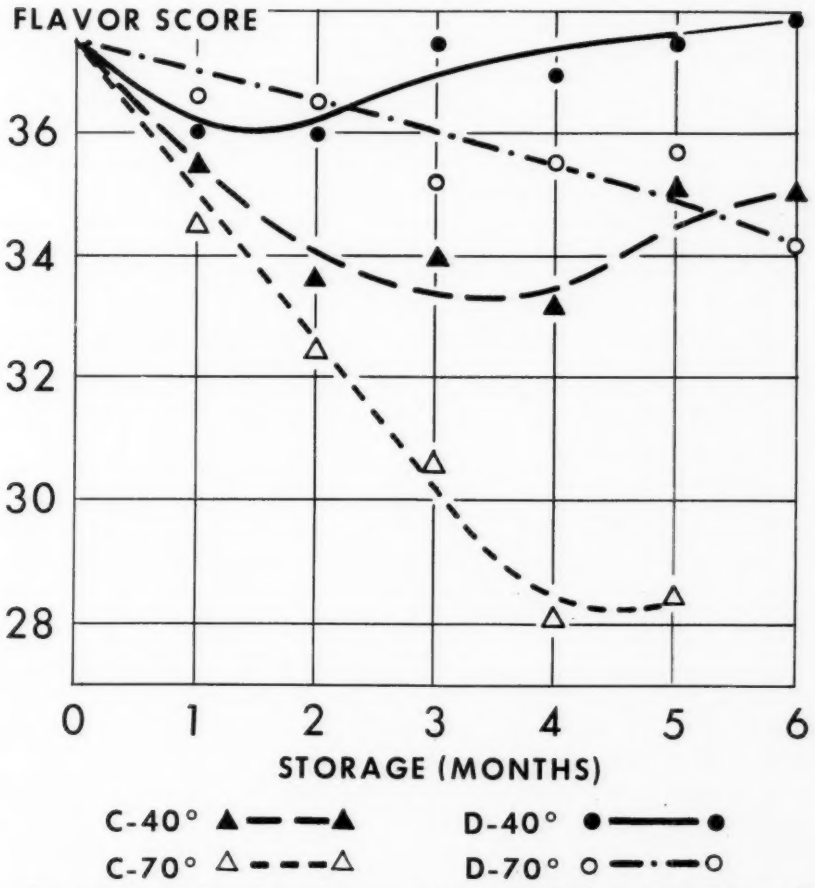

FIG. 4. Effect of deaerating and vacuum-packing concentrated sweetened cream on its flavor score during storage at 40 and 70° F. C = Undeaerated, D = Deaerated and vacuum-packed.

exemplified in Figures 1, 2, and 3. In Figure 1, the temperature of the blend when it was flashed into an unheated vacuum pan was 135°, in Figure 2 it was 150°, and in Figure 3, 170° F. These results are based on flavor score averages of two or more experiments at each of the three pumping temperatures.

When deaeration was initiated at 135° F., and with no additional heating in the vacuum pan, there was a decrease in flavor score of nearly two points at the end of 6 mo., when the product had been held under 29 in. of vacuum for 60 min. and stored at 40° F. On the other hand, when the concentrated sweetened cream was delivered to the vacuum pan at 150° F., only a brief holding period was sufficient to cause the product to have a somewhat better flavor after 6 mo. at 40° F. than that deaerated at 135° F. And when deaeration was initiated at 170° F. and was continued for 60 min., the product retained its flavor as well at 70° as did the comparable samples of Figure 1 that were stored at 40° F. and in spite of the fact, as these figures show, that the flavor of concentrated sweetened cream is much more stable at 40 than at 70° F.

The effect of deaeration is shown further in Figures 4 and 5. The data are based on average scores and peroxide values of two similar experiments in which deaeration consisted of flashing the concentrate into the vacuum chamber at about 150° F. and holding it continuously under a vacuum of 29 in. for 1 hr.

The peroxide values of the nondeaerated (control) samples increased rapidly at both storage temperatures. At 70° F., the flavor scores decreased steadily to 28 and at 40° F. they dropped to 33.5 in 2 mo. and remained between 33 and 35 during the remaining 4 mo.

The peroxide values of deaerated samples stored at 40° F. increased slightly in the first 2 mo., then decreased in the 3 mo. following. At 70° F. there was an irregular and small decrease in peroxide values. The irregularities in these curves may have been due to lack of sensitivity in the method at such low peroxide concentrations.

Control samples stored at 70° F. for 2 mo. had a slight to pronounced oxidized flavor according to five of the six judges. One of the samples stored at 40° F. for 2 mo. had a slight to definite oxidized flavor according to four judges.

The initial flavor score of the deaerated and vacuum-packed samples stored at 70° F. was retained well for 2 mo., but it decreased to 34 during the ensuing 4 mo. At 40° F., the initial score was maintained more than 6 mo., the score being 38 at 7½ mo.

One deaerated sample stored at 40° F. for 4 mo. had a slight oxidized flavor according to three judges, but no such flavor was found at 5 mo. After 5 mo. one deaerated 70° sample was criticized by one judge for a slight, and by a second for a definite, oxidized flavor.

Oxygenation. Bubbling oxygen into concentrated sweetened cream for 1 hr. at 105-110° F. resulted in a marked increase in the peroxide values and decrease in flavor scores during storage for 3 mo. In 1 mo., oxygenated samples stored at 70° F. had a peroxide value of 1.58, whereas that of the control was 0.56. After 2 mo., the peroxide values were 3.85 and 0.98, respectively. The 40° F. samples had correspondingly similar but lower peroxide values; within 2 mo.

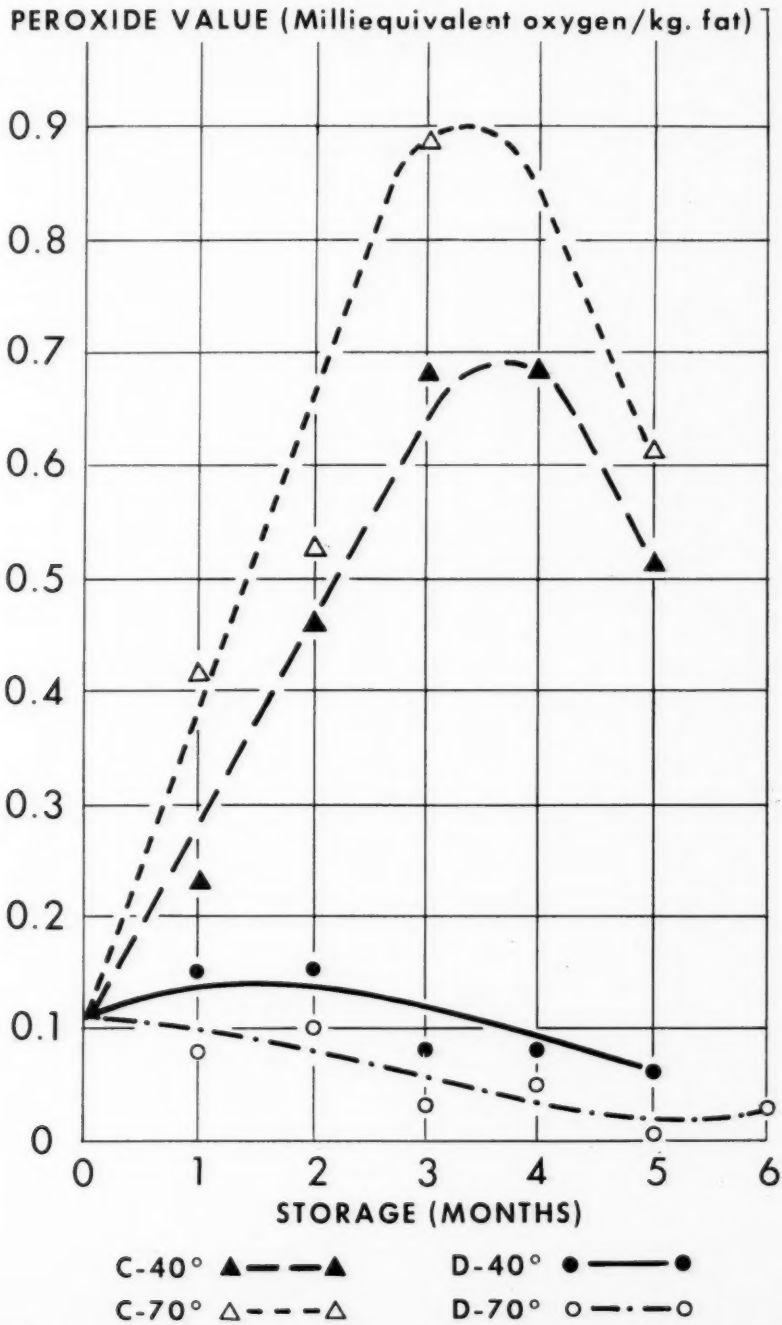

FIG. 5. Effect of deaerating and vacuum-packing concentrated sweetened cream on its peroxide value during storage at 40 and 70° F. C = Undeaerated, D = Deaerated and vacuum-packed.

the peroxide value of the oxygenated sample was 1.49 and the control was 0.64.

The oxygenated samples stored at 70° F. developed an objectionable flavor within 1 mo.; at 40° F. they maintained their flavor for only 1 mo., then their flavor deteriorated rapidly.

ACKNOWLEDGMENT

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OCCURRENCE OF 5-HYDROXYMETHYLFURFURAL IN VACUUM FOAM-DRIED WHOLE MILK AND ITS RELATION TO PROCESSING AND STORAGE

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SUMMARY

The relationship of 5-hydroxymethylfurfural (HMF) and its precursors to flavor storage stability was investigated. Vacuum foam–dried whole milk was produced and stored at 73° F. HMF values were determined initially, and were found to range from ~ 1-5 μM/liter. Flavor stability, as a function of storage time, was determined by comparison to freshly dried powder in a Rank Paired Comparison test. A significant relationship was found between flavor stability and the initial HMF value.

Formation of HMF during drying was studied as a function of maximum drying temperature. Twenty-five runs were made, wherein the maximum foam temperature was varied from 103 to 200° F. An Arrhenius-type equation was found to correlate rate of formation of HMF with maximum drying temperature. Analysis of variance showed this relationship to be highly significant.

The flavor of dry whole milk during processing and storage is subject to degradation by many means, for the most part poorly understood. Patton, in a review article, gives Maillard browning of lactose in milk as one of these pathways (4). He also has shown that hydroxymethylfurfural (HMF) is produced by heating lactose with casein (3). Hodge reviews a scheme whereby the Amadori rearrangement can produce HMF and its precursors (1). Keeney and Bassette, in a study of HMF in instantized and noninstantized nonfat dry milk, developed a digestion method which detects total HMF [free HMF and its precursors (2)]. They found that high values of HMF by this test were coincident with off-flavors, particularly cereal, in the skim powders. They suggest determination of HMF as a means of detecting early stages of the browning reaction.

It is the purpose of this paper to show a relationship between HMF, as measured by Keeney and Bassette's analysis, and the storage life of dry whole milk. It is further intended to relate this analysis to process conditions during drying of the whole milk and thence to storage stability.

EXPERIMENTAL PROCEDURES

Production of dry whole milk. Dry whole milk was produced by the method of Sinnamon *et al.* (5). Fluid whole milk is concentrated to 45-48% solids in a falling-film, recirculation type, vacuum evaporator, homogenized in one pass at 3,000 p.s.i. and for 1 to 1½ min. at 500 p.s.i. while metering in finely dispersed N_2. The gassed concentrate is chilled, flowed into trays, and placed in

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a vacuum shelf dryer. A vacuum is applied, resulting in a rigid milk foam structure, which is then dried. Temperature control is achieved by following the foam-tray boundary temperature with a thermocouple at that point. Normal production runs are made at a boundary temperature which reaches a maximum and a plateau at ~ 110° F. Drying time ranges from 1½ to 4 hr. The time is a function of foam height, concentrate solids, and terminal moisture.

Determination of HMF. HMF was determined by the Method B of Keeney and Bassette (2), with some modification. It was found necessary to cool the test tube tops to insure complete condensation of the water vapor. This was accomplished by constructing a water bath having a metal plate cut to serve as a test tube holder and a steam barrier. The height of the shield was set so that the digestion mixture in the test tube was submerged and just below the metal shield. The portion of the test tube above the shield was cooled by directing a fan across the bath. Powders were reconstituted to a total solids equivalent to that in the starting milk, i.e., 12%. Digestion was begun within 5 min. of reconstitution. The method yields HMF values with the absorbance of raw milk as a reference point (equal to 0).

References made hereafter to HMF refer to the results of this analysis.

Organoleptic analysis. A typical test used in our storage stability studies is designed to show the effect of some processing or packaging treatment. Thus, two samples, a control and a treated variable, are stored. Organoleptic analysis is by the Rank Paired Comparison Test (RPC), as described by Terry, Bradley, and Davis (6). In this test three or more samples are compared by presenting them in all combinations as pairs to a taste panel; the pairs are presented separately. Even though there are three or more samples tested, valid comparisons between any two can be made. In our studies, three samples are tested; a freshly dried control, a stored control, and a stored variable. The data used in this study represent only a comparison between the stored control and the fresh control. It is not within the scope of this paper to treat the effect of the process or packaging variables.

The taste panel for these tests numbers 15 to 20 judges, who were previously screened and trained for taste acuity. The criterion is, "Which sample of each pair tastes more like fresh milk?" Statistical analysis is by analysis of variance. Results of the RPC tests are expressed as fresh control is fresher than the stored sample at X% significant level of difference (SLD). SLD = 100% represents no difference in the two samples, whereas SLD = 0.1% represents a marked difference.

Storage studies. Powder was dried in the manner described above to a moisture content of ~ 2-3%. It initially had consistent good flavor and physical properties. The powder was analyzed for HMF, packaged in N_2, 0.1 to 0.5% O_2 in the can's headspace, and stored at 73° F.

Samples of the stored powder were withdrawn at intervals and tested organoleptically.

Drying temperature studies. Whole milk powder was produced in the manner described, except that maximum foam temperature, T_m, (normally that at the

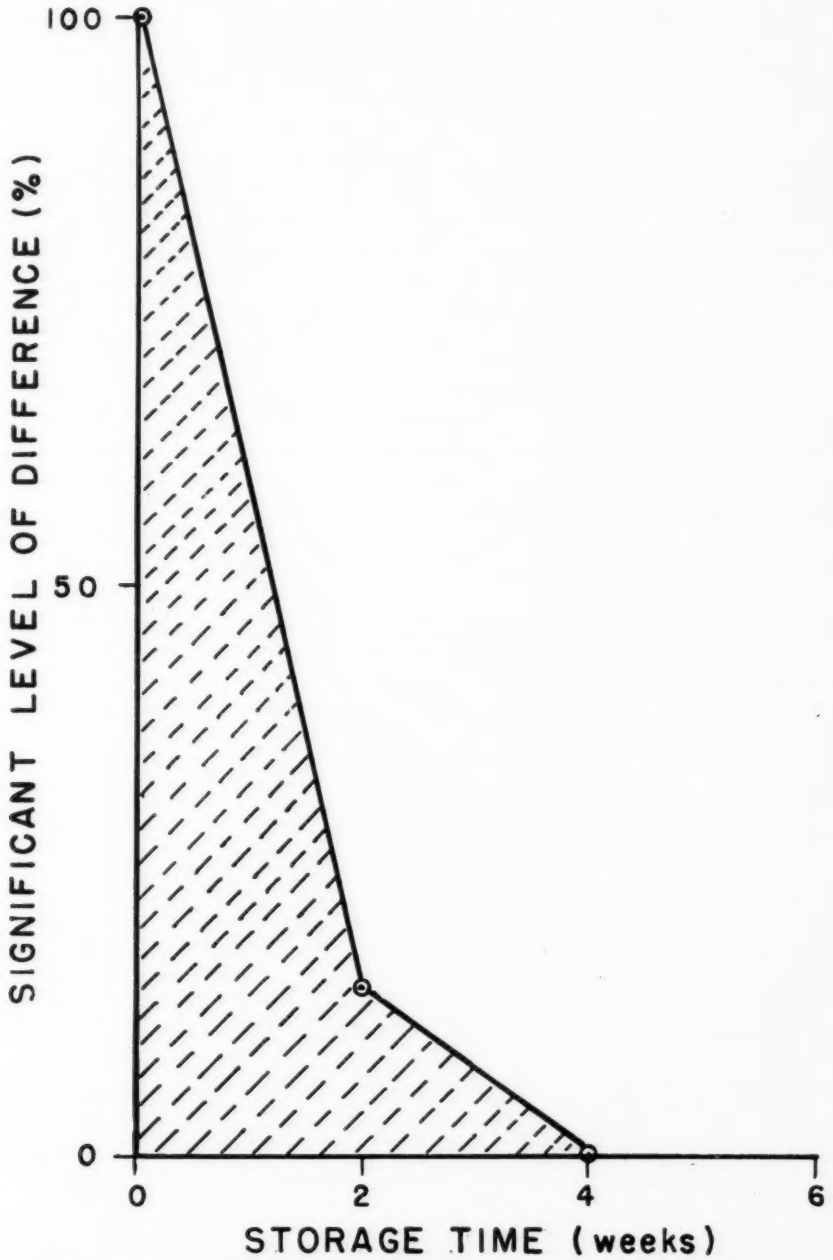

FIG. 1. Example of data from rank paired comparison test as significant level of difference between stored sample and fresh control vs. storage time. Shaded area represents Storage Stability Index (SSI).

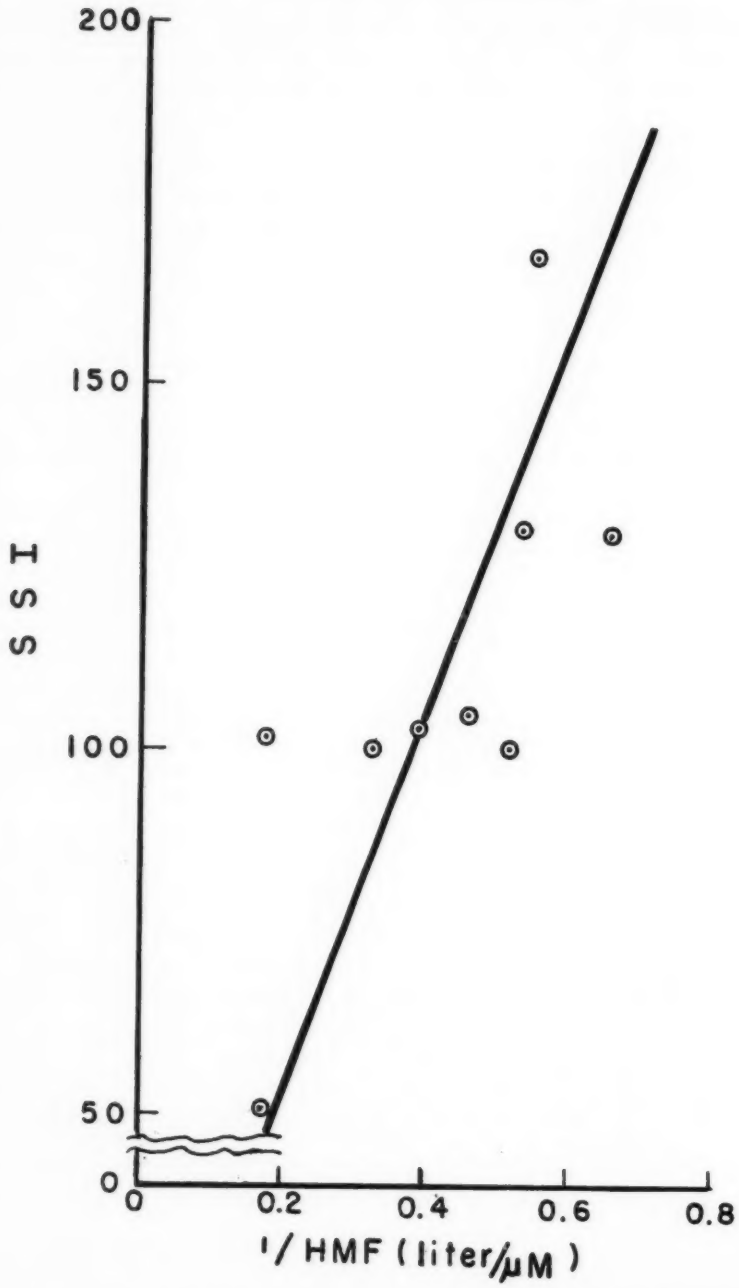

FIG. 2. Storage Stability Index (SLD × storage time) vs. $\frac{1}{\text{HMF}}$ for stored controls at zero time.

foam-tray boundary) was varied. T_m ranged from 103 to 200° F. HMF analyses were made on the freshly dried samples. These runs were made during January-March, 1959, and January-February, 1961.

RESULTS

Storage. Nine storage tests were made. For each, results of the periodic RPC tests were plotted as per cent Significant Level of Difference between freshly dried control and stored control vs. time. An example of this is shown in Figure 1. To quantitatively express the relative storage stability of these powders, the area included in this plot between $SLD = 100\%$ and $SLD = 0.1\%$ was taken to be an index of storage stability (SSI). One can observe from Figure 1 that a powder of greater stability will take longer to reach $SLD = 0.1\%$ and result in a greater area. In the nine storage tests $SLD = 0.1\%$ was reached in from 1 to 12 wk., depending on the rate of formation of off-flavors.

SSI was plotted against the reciprocal of initial HMF for nine stored controls (Figure 2). An analysis of variance showed this relationship to be significant ($p \cong 0.025$).

Drying temperature. Twenty-five drying runs were made for the temperature study. The commonly used method for expressing the influence of temperature on reaction velocity is that of Arrhenius.[2] The usual plot is the log k vs. $1/T$. If we may describe the reaction by an irreversible rate equation $r = k\ (C_r - C_p)^n$,[3] an approximately linear relationship between k and r is to be expected when C_r is much greater than C_p. This assumption is justified because the concentration of lactose or casein is of the order of 10^4 to 10^6 times the concentration of the product (HMF). Thus, the data can be treated by plotting log r vs. $1/T_m$.

Five of the 25 runs yield zero or negative rates of formation of HMF, where $r = \text{rate} = \dfrac{[HMF \text{ (dried powder)} - HMF \text{ (feed milk)}]}{\text{Drying time}}$. Accordingly, the remaining 20 points were plotted as above (Figure 3). This relationship was analyzed statistically by an analysis of variance and found very highly significant ($p < 0.001$). The regression coefficient was -4.398 and it was assumed that the slope corresponded to E/R as defined by the Arrhenius equation. In

[2] The Arrhenius equation is frequently shown as:

$$k = Ae^{\frac{-E}{RT}}$$

where;

k = rate constant
A = constant, referred to as frequency factor
E = Activation energy
R = Gas constant
T = Temperature, absolute

See, for instance, Smith's Chemical Engineering Kinetics, McGraw-Hill. 1956.

[3] C_r = conc. reactant at zero time
C_p = instantaneous conc. product formed
$n = 1, 2, \ldots$

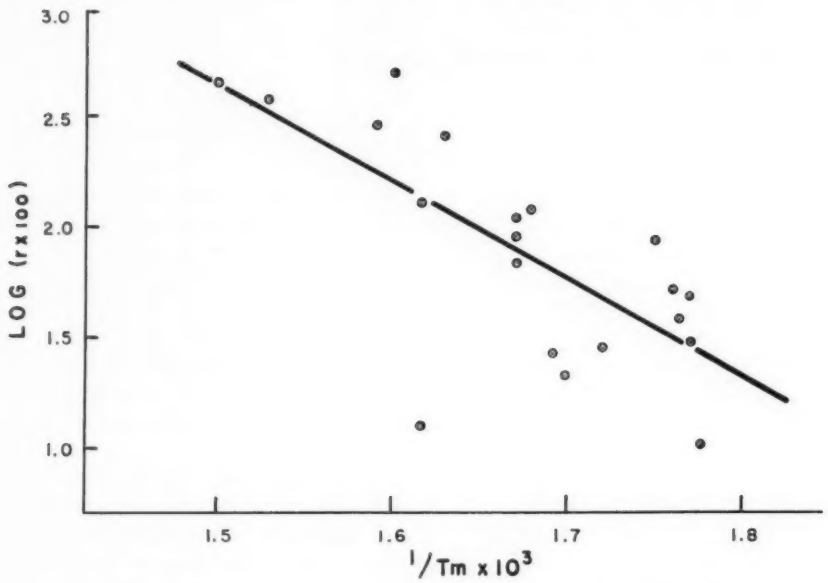

FIG. 3. Log ($r \times 100$) (rate of formation of HMF during drying) vs. $\frac{1}{T_m}$ maximum foam temperature. r is expressed in μM/liter-hour, and T_m in °R.

order to consider all of the drying runs, this value was substituted and the twenty-five points plotted as r vs. $e^{\frac{-E}{RT_m}}$ (Figure 4). This relationship was also analyzed statistically and found much more significant than that in Figure 3.

DISCUSSION

The RPC test is quite sensitive, but nonselective with respect to off-flavors. One should note that a powder significantly less fresh than the fresh control at the 0.1% level is not necessarily unacceptable. However, this is a good means of detecting early storage off-flavors in the powder.

Statistical analysis of duplicates of HMF run on these powders showed an error of ± 0.8 μM/liter at the 95% confidence level. With HMF values of about 1-5 μM/liter, this provides a fair amount of scatter, and is the most plausible reason for the zero and negative rates discussed earlier, as these values fell within experimental error.

The plot, Storage Stability Index (SSI) vs. $\frac{1}{\text{HMF}}$ (Figure 2) is sufficient to show qualitatively the relationship between storage stability and the initial HMF of the product within the range of 1 to 5 μM/liter. Thus, the lower the HMF content the more stable the product.

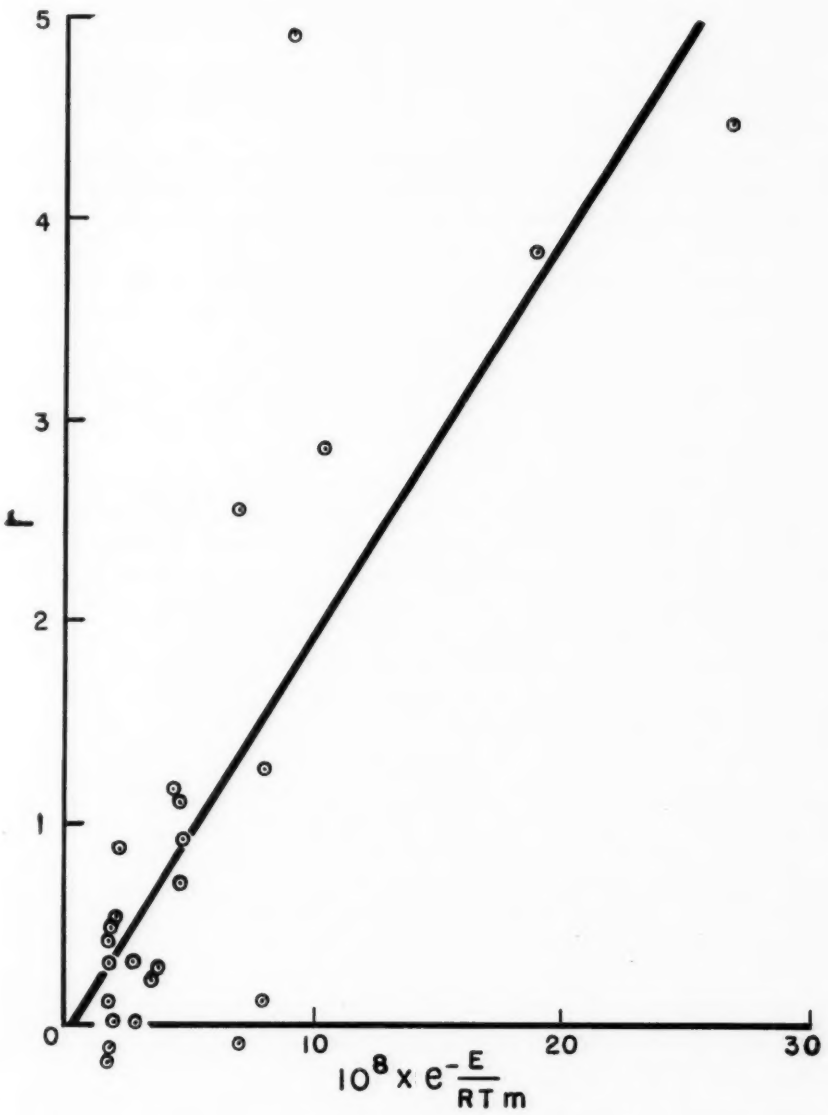

FIG. 4. r (rate of formation of HMF during drying) vs. $e^{\frac{-E}{RT_m}} \times 10^8$. T_m is maximum foam temperature and E/R was calculated from slope of plot in Figure 3.

This may indicate that the HMF analysis measures the potential for production of browning off-flavors on storage or HMF may simply be an index of heat

exposure during processing and thus indirectly indicates the initiation of nonbrowning reactions which produce off-flavors.

From Figures 3 and 4, HMF has been shown to be a good index of heat exposure and, given an HMF change during drying, one may estimate the time-temperature relationship.

As further substantiation of the assumptions, one may note that, although the expected point (0, 0) was not considered in the statistical analysis of Figure 4, extrapolation of the derived relationship to $T = 0$ gives virtually a zero rate of formation.

An activation energy, E, could be calculated from Figure 3, but may be of little meaning because it would be for varying amounts of products of three consecutive reactions. Therefore, it is not presented.

Drying times used in the calculation of rates were from time of application of heat to the foam to time of cooling the dried foam before removal. This is the more convenient variable, but some variation will exist between this and time-at-T_m.

A firmer basis for relating rate of formation of HMF to drying temperature would be to use the local temperature in the foam, or an average temperature integrated over the foam volume and time. However, either of these would be quite difficult to observe or estimate and would be of less practical value as a control parameter. This does indicate that a variable relationship between T_m and T (local) should contribute to a greater dispersion on the plot.

An additional variation should be due to change in HMF during the evaporation of the fluid milk. This, however, is done at 88-90° F. and fairly short times, so that variation is assumed small.

CONCLUSIONS

An estimate of the flavor stability of a number of foam-dried whole milk powders could be made from the HMF analysis at zero time.

A relationship was found between rate of formation of HMF and maximum foam temperature during drying, showing this analysis to be a good index of heat exposure. This should aid in establishing process conditions which will maximize flavor stability in the whole milk powder.

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SIZE OF PROTEIN PARTICLES IN ULTRAHIGH-TEMPERATURE STERILIZED MILK AS RELATED TO CONCENTRATION

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SUMMARY

Ultracentrifugation studies of milk sterilized by ultrahigh temperatures have shown that sedimentation constants increased. Sedimentation studies of reconstituted concentrated milks showed that milks reconstituted immediately after concentration had sedimentation constants approximately the same as the milks after preheating. Constants of the same milks reconstituted after sterilization were much higher. This study was undertaken to determine the relationship between concentration and size of particle after sterilization at a given temperature. To minimize the interference of fat in the determination of sedimentation constants, fat was removed by centrifugal separation of the raw milk at 100° F. Preheating (180° F., 4.16 sec.) and sterilization (293° F. no hold) temperatures used had been found to cause minimum clustering of protein particles. Portions of the preheated milk were concentrated 3:1, 2:1, and diluted one-third with deionized water. Logs of the sedimentation constants of the reconstituted sterile concentrated milks and sterile normal and diluted milks, when plotted against concentration, formed a straight line which intersected the zero concentration axis at the log of the sedimentation constant of the smallest micelle in raw skimmilk, reported by Ford and Ramsdell (1). The relationship of the particle size to sedimentation constant can be expressed by the equation $\frac{d^2}{S} = \frac{18\eta}{\rho - \rho_0}$

The effect of heat stability of milk proteins on the quality of dairy products is complex and variable (2). Ultrahigh-temperature sterilization has eliminated caramel and associated flavors of conventional evaporated milk, but it has increased the problem of thickening and gelation. Sedimentation and microscopic studies of sterile concentrated milks have shown that thickening and gelation involve changes in particle size and aggregation (5).

The size of the particles of normal milk increased as the temperature was raised and clusters finally formed. The point of cluster formation varied with different milks. The particles in reconstituted milks direct from the vacuum pan were the same size as they were in the preheated milks before concentration, but after sterilization the particles were many times larger (5).

The purpose of this investigation was to study the relation between the size of particles and the concentration of solids in sterile concentrated milk.

EXPERIMENTAL PROCEDURE

Mixed raw milk was centrifugally separated at 90 to 100° F. to obtain a milk with 1.36% fat. After heating the milk in the Mallory heat exchanger at 180° F. for 4.16 sec., it was divided into four portions and treated as follows: (1) Diluted to two-thirds of normal composition with deionized water; (2) normal composition; (3) vacuum concentrated to a ratio of 2.13:1; (4) concentrated

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to a ratio of 2.99 : 1. The milks were not standardized for solids content after removal from the vacuum pan. All portions were sterilized at 293° F. ± 1° with no intentional holding time. Total heating time was 6.24 sec.

Sterile concentrated Milks 3 and 4 were diluted to the composition of normal Milk 2, and sedimentation constants were determined as previously described (4). The sedimentation constant of Milk 1 was corrected for the viscosity and density of solution. Hydrogen ion activity was always higher in concentrated milks, but the pH after sterilization plotted against concentration of solids was not a straight line. To determine the probable effect of lowered pH on the size of protein particles after sterilization, the pH of centrifugally separated skimmilk was adjusted by adding dilute acetic (0.1 *N*) and phosphoric (0.7 *N*) acids as shown in Table 1. Sterile milks were examined microscopically and photomicrographs were made. Nitrogen analyses were made by the semimicro method.

TABLE 1

pH after sterilization of milk, dilute, normal, and concentrated, and skimmilks acidified with dilute acetic or phosphoric acid

	pH
Milk diluted ⅓	6.75
Milk (1.36% fat)	6.65
Concentrated 2.13 : 1	6.38
Concentrated 2.99 : 1	6.30

Acidified skimmilks

Acetic (0.1 *N*) % Added			Phosphoric (0.7 *N*) % Added		
Water	Acid	pH	Water	Acid	pH
8	0	6.58	5	0	6.62
0	0	6.57	0	0	6.63
6	2	6.49	3.75	1.25	6.53
4	4	6.40	2.5	2.5	6.45
2	6	6.31	1.25	3.75	6.30
0	8	6.22	0	5.0	6.13

RESULTS

As shown in Figure 1, the logarithms of the sedimentation constants $\times 10^{13}$, when plotted against the ratio of concentration, formed a straight line, and when extrapolated, intersected the zero concentration axis at the log of the sedimentation constant of the smallest casein micelle reported by Ford and Ramsdell (1). Additional similar experiments gave the same results.

In Figure 2, Table 2, the logs of the sedimentation constants of the acidified skimmilks plotted against pH are compared with those of the reconstituted concentrated milks. The milk to which acetic acid was added to adjust the pH had less than 25,000 bacteria per milliliter by direct count and more than 2,000,000 leucocytes per milliliter. The milk to which phosphoric acid was added had less than 10,000 bacteria and 100,000 leucocytes per milliliter. At the first additions of acids, the sedimentation constants paralleled those for the

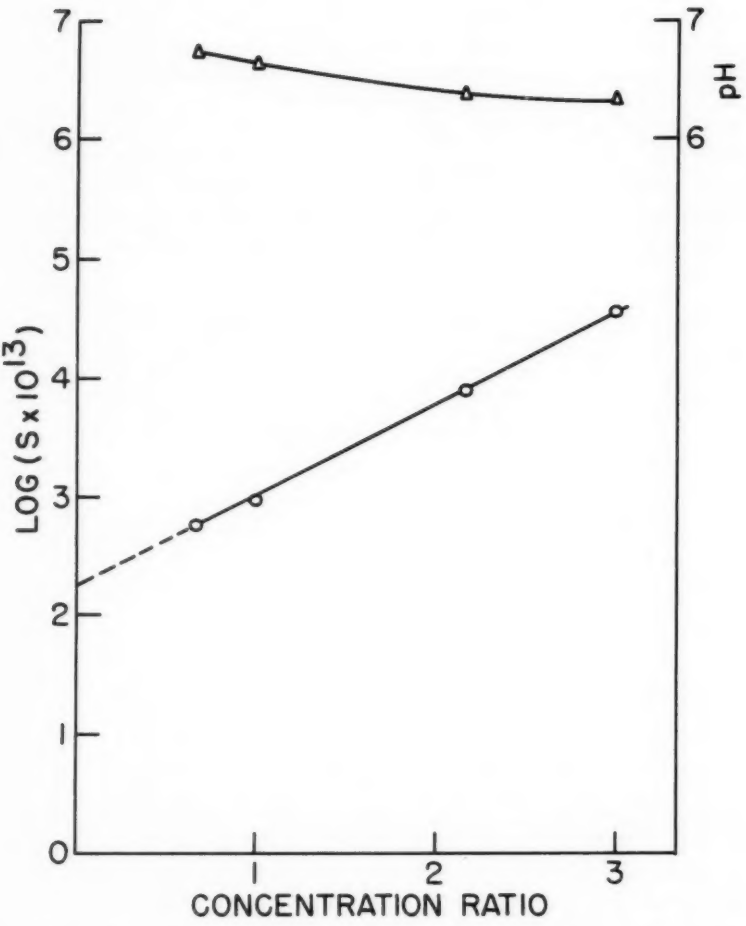

FIG. 1. Logs of sedimentation constants of sterile normal milk, same milk diluted, and concentrated (○) and pH measured after sterilization (△) vs. ratio of concentration.

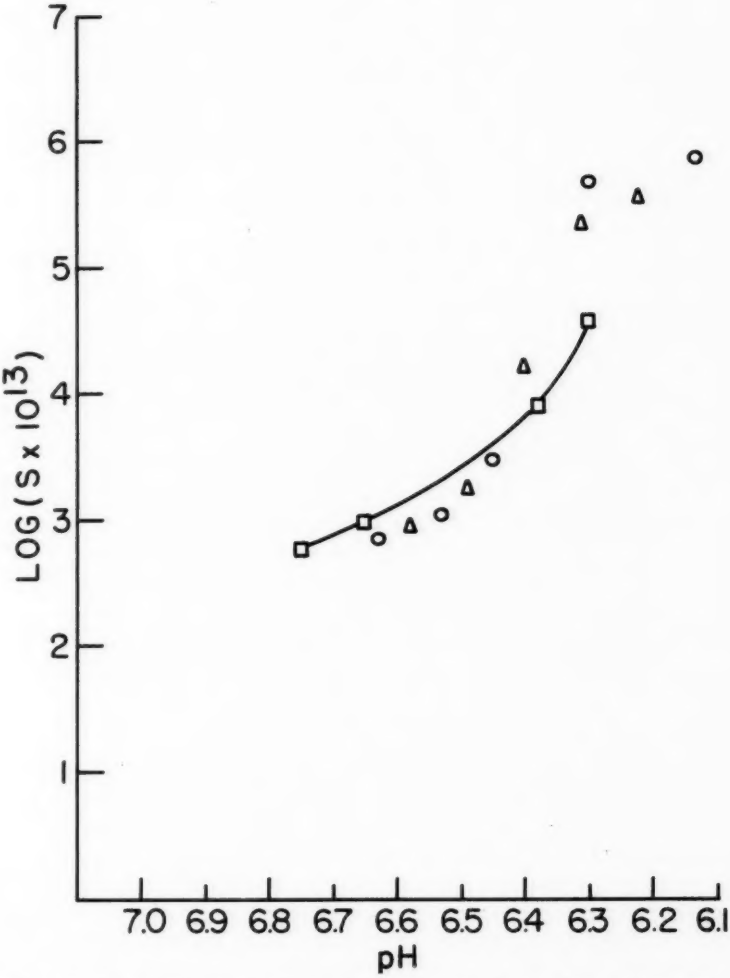

FIG. 2. Logs of sedimentation constants of milks described in Table 1, vs. pH: normal milk and concentrated milk (□), skimmilk acidified with acetic acid (△), and skimmilk acidified with phosphoric acid (○).

TABLE 2

Sedimentation constants and logs of sedimentation constants ($\times 10^{13}$) of sterile milks and acidified milks

Milks [a, b]			Skimmilk + acetic acid [b]			Skimmilk + phosphoric acid [b]		
pH	$S \times 10^{13}$	Log	pH	$S \times 10^{13}$	Log	pH	$S \times 10^{13}$	Log
6.75	560	2.74	6.58	860	2.93	6.62	690	2.84
6.65	970	2.98	6.57	960	2.98	6.63	690	2.84
6.38	7,800	3.89	6.49	1,700	3.23	6.53	1,085	3.03
6.30	36,000	4.55	6.40	16,000	4.20	6.45	3,000	3.47
			6.31	220,000	5.34	6.30	450,000	5.65
			6.22	340,000	5.53	6.13	680,000	5.85

[a] Data plotted in Figure 1.
[b] Data plotted in Figure 2.

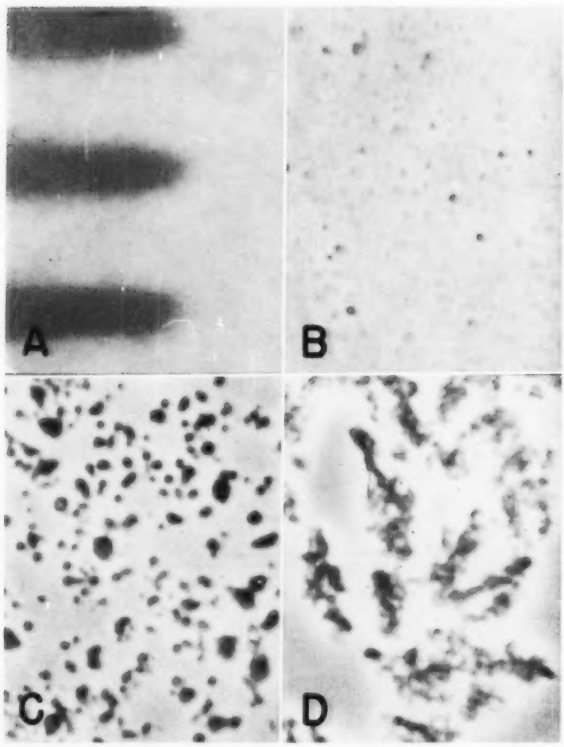

FIG. 3. Photomicrographs of: A. Stage micrometer, 10 μ between marks. B. Skimmilk heated 180° F. for 4 sec. C. Milk concentrated 2.99: 1 and sterilized 293° F., pH 6.30 after sterilization. D. Skimmilk acidified with acetic acid and sterilized at 292° F., pH after sterilization 6.31.

concentrated milks, but the particles clustered at a higher pH in the acidified milks than in the concentrated ones. The milk concentrated 2.99:1 had a pH of 6.30 and the largest clusters in the acidified milks at that pH were 15 μ long and settled out on standing. They were elongated and made up of straight chains of discrete particles (Figure 3).

From the nitrogen analyses, the per cent of serum proteins denatured in the acidified skimmilks was variable in about the same range as the concentrated milks (Table 3).

DISCUSSION

The results reported in sedimentation constants can be converted to size of particle by the form of Stokes law $\frac{d^2}{S} = \frac{18\eta}{\rho - \rho_0}$ in which d is the diameter of particle, S the sedimentation constant, η the viscosity, and $\rho - \rho_0$ the difference in density between the particle and solution. The viscosity and density of the solution were controlled by diluting the concentrated milks at the time of centrifugation. If the density of the particle could be considered constant, the square of the diameter would be directly proportional to the sedimentation constant. The shape and structure of the clusters in milks acidified by the two acids were similar. They were different from the shape and structure of clusters in other concentrated milks of the same pH which had been heated sufficiently to cause extensive clustering.

TABLE 3

Per cent of serum proteins denatured [a] in sterile milk and skimmilk acidified with phosphoric acid

Unacidified milks, Table 1		Acidified skimmilk	
pH	% Denatured	pH	% Denatured
6.75	47.36	6.63	48.38
6.65	35.58	6.62	45.67
6.38	52.14	6.53	54.72
6.30	43.72	6.45	50.20
		6.30	54.72
		6.13	45.67

[a] Nitrogen analyses were made by the semimicro method.

An evaluation of the reasons for this difference in structure and shape of clusters is difficult. The greater viscosity of the concentrated milks would tend to retard the formation of clusters. Even though the difference in voscosities at sterilization temperature would be much less than at room temperature, it could affect the cluster formation. It is possible that some of the difference could be due to the difference in ions present as found by Zittle and Pepper (6), and Tessier and Rose (3) in model systems. Nitrogen analyses (Table 3) did not reveal a correlation between the per cent of serum protein denatured and change in size of particles and clustering. A possible explanation of the variations in proteins denatured could be that temperature and time are at a critical point in protein denaturation and the variations reflect minor fluctuations in temperature.

A possible explanation of this complex relationship in the increase in size of protein particles at sterilization temperatures involves: (a) Partial removal of calcium and phosphate from solution (2) and deposition on surfaces such as bacterial cells, leucocytes, and sediment (4), with opening of some reactive sites on casein micelles; (b) at sterilization temperatures viscosity was decreased and the speed of reaction increased many times above that at room temperature; (c) as particles became larger both positive and negative sites attracted other ions, so that the particles grew at an increasing rate and; (d) partial denaturation of serum proteins with complexing and increase in size of casein micelles or other materials, or the formation of new particles composed entirely of serum proteins. Throughout our experiments we have evidence that the denatured serum proteins sediment with the casein in ultracentrifugation.

The preheating treatment, employed in conventional evaporated milk to promote stability in retort sterilization, caused the formation of clusters in some but not in all milks. These clusters increased viscosity and in ultrahigh-temperature sterilization resulted in particles large enough to cause graininess. Preheating and sterilization of skim and concentrated skim at temperatures and times to cause a minimum of clustering have resulted in sedimentation constants which indicated a systematic increase in size with concentration. The joining of discrete particles into straight chains before the formation of clusters in acidified milks suggested a specific bonding, as opposed to random aggregation of irregular clusters in milks and concentrated milks when heated to higher temperatures or held for longer times. The straight chains were similar in microscopic appearance to the straight chains found in the initial stages of gelation of sterile concentrated milks stored at 100° F. The clusters in the acidified milks were different from the gel structure, in that microscopic examination revealed partial dissociation of the clusters in acidified milks to straight chains after storage at 100° F.

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DETERMINATION OF β-LACTOGLOBULIN IN COMPLEX SYSTEMS BY A SIMPLE IMMUNOLOGICAL PROCEDURE [1]

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SUMMARY

A simple method, based on immunological procedures described originally by Oudin, has been developed to quantitate the amount of β-lactoglobulin present in complex systems. The solution containing β-lactoglobulin is allowed to diffuse into an agar layer containing antisera to the β-lactoglobulin. The distance of this migration, as measured by the formation of a turbid zone in the tube, is proportional to the β-lactoglobulin concentration. The usable range of the method is about 10-80 μg/ml. Since milk contains about 2,500 μg/ml of β-lactoglobulin, dilution is the only sample preparation required for milk and liquid milk products. The β-lactoglobulin in solid materials must first be solubilized in a suitable tissue homogenizer.

A concentration procedure is also described with which it has been possible to determine less than 1 μg/ml of β-lactoglobulin in a complex nutrient medium used to maintain bovine mammary secretory cells in laboratory culture and containing levels of other proteins up to ten-thousand times more concentrated than the β-lactoglobulin.

The use of more advanced techniques during the past few years has indicated that the classical protein fractions of milk are composed of several different protein entities. In particular, the technique of electrophoresis by the moving boundary U-tube procedure provided the means by which several of these proteins were originally identified, and this subsequently led to their isolation and characterization (3, 7, 17). Several of these electrophoretic fractions have now been shown to be heterogeneous (3, 7). Even though other methods have been developed to determine some of the milk proteins, for several of the proteins the electrophoretic procedure is still the only method that has been described to evaluate them quantitatively. The intricacies and/or the inherent shortcomings in all of these methods, however, has made it desirable to find more simplified and suitable methods to determine the individual proteins of milk. This is especially pertinent where it is desired to evaluate and quantitate the presence of an individual protein in a system where the protein is present in extremely low concentration, both in terms of an absolute level and relative to the levels of other proteins present. Such a problem was faced in an investigation of protein synthesis in the mammary secretory cell in tissue culture (5, 6), where it was desired to evaluate the synthesis of microgram quantities of β-lactoglobulin present in a complex mixture of nutrient materials containing other proteins at levels up to over ten-thousand fold more concentrated than the β-lactoglobulin. The present communication describes a quantitative method which has been used to determine β-lactoglobulin in concentrations of less than 1 μg/ml in this complex system, and is adapted from an immunological procedure originally described by Oudin (14).

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Upon the development of this procedure, however, it became apparent that the method was readily applicable to the determination of β-lactoglobulin in milk. The method not only possesses many advantages in utility over any method previously described [see (17) for review], but because of its sensitivity also allows the determination of β-lactoglobulin in products made with milk for which no method has been available.

MATERIALS AND METHODS

Antisera to β-lactoglobulin.[2] β-Lactoglobulin was prepared for the antigen by isolating β-lactoglobulin from mixed herd milk and recrystallizing it eight times (11). It was injected into several rabbits both intramuscularly as a 1% emulsion in mineral oil and intraabdominally and intravenously as a 1% solution in saline until suitable blood titers were attained. The rabbits were then bled; serum was prepared from the blood which was pooled and stored at −18° C. in small vials.[3]

Agar. This was prepared as a 1% solution by dissolving 5.0 g. of agar (Difco), 4.25 g. of NaCl, and 100 mg. of merthiolate (Lilly) in about 450 ml. of boiling distilled water. The hot solution was filtered (Whatman No. 1 paper), cooled to 50° C., adjusted to about pH 7 with 1 *N* NaOH, and brought to 500 ml. total volume. The solution was then dispersed into tubes and stored at 4° C.

Tubes. Glass tubing with an inside diameter of 2.0 mm. was cut into 10-cm. lengths, cleaned, dried, and one end sealed in a flame.

pH 6.6 diluting buffer. Where not otherwise specified, this buffer was used for dilutions. The buffer was composed of 0.0021 *M* K_2HPO_4, 0.0039 *M* KH_2PO_4, and 0.0139 *M* NaCl. The buffer was stored in the refrigerator for periods not exceeding 2 wk. and was routinely prepared by diluting 8.1 g. of NaCl together with 10 ml. of a 100-times concentrated phosphate buffer (preserved with chloroform) up to one liter with water.

Procedure. The diagram in Figure 1 shows the physical aspects of the assay procedure. The antisera-agar solution was prepared by mixing equal volumes of antisera and melted agar. The agar was first heated to boiling to liquefy it and then cooled to 45° C. before mixing with the antisera. Immediately, using a 1-ml. syringe and a 5-in. needle (No. 20), the antisera-agar solution was placed in the bottom of the tubes to a depth of about 3-5 cm., allowing no bubbles to form in the process. After cooling at room temperature until the agar was solidified, the antigen solution was placed over the antisera-agar layer with a syringe and needle to a depth of about 3 cm., being careful to leave no bubbles

[2] The antiserum to β-lactoglobulin is the only item of the reagents and equipment which is not readily available. The Nutritional Biochemicals Corporation (Cleveland 28, Ohio) expects to have this item available commercially in the near future.

[3] The serum was prepared from the blood in the usual manner, centrifuging it after clot formation had taken place. The pooled serum when diluted 1:1 with saline gave a barely discernible reaction in precipitin tests (10) to a β-lactoglobulin solution (in saline) diluted to 0.3 μg. per milliliter or milk or milk whey diluted 1:10,000. It should be noted that this is not a high titer serum.

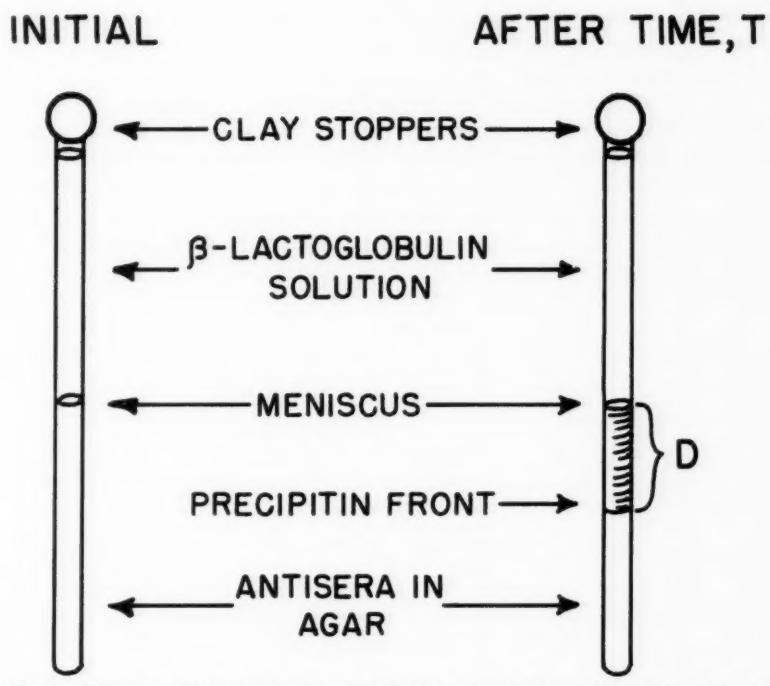

FIG. 1. Diagram of the assay tubes. The initial tube is set up with a layer of solution containing β-lactoglobulin (concentration = C) over a layer of agar containing antisera to β-lactoglobulin. The clay stopper prevents evaporation loss and the tubes are placed upright in a rack and maintained at the controlled temperature.

at the interface. The tops of the tubes were sealed with wet clay and the tubes placed upright in an incubator at 37° C. At appropriate intervals the length of the turbid zone in the agar was evaluated with the use of a vernier caliper to the nearest 0.1 mm. The measurements were made from the bottom of the meniscus to the precipitin front.

Standard curve. The standard curve was constructed using varying levels of β-lactoglobulin dissolved in the pH 6.6 diluting buffer. Oudin (14) originally noted that the distance (D) of migration was linear with the square root of time ($\sqrt{T}$) for any given antigen level. It has been noted in the present investigation that there appears also to be a linear relationship between the square of the distance (D^2) and the β-lactoglobulin concentration (C) for any given time up to about the 80-μg. level. This plot is shown in Figure 2, and it has been found convenient to use it as a quick reference check. The plot in Figure 2, however, is dependent on readings made at definite time intervals. Thus, the relationship was developed which is shown in Figure 3, plotting the distance divided by the square root of time ($D/\sqrt{T}$) against the logarithm of the β-lactoglobulin concentration (C). It should be noted that as low as about 8 μg. can be evaluated;

however, the most usable range is from 15 up to about 70 μg., and the level in a single sample in this range can be evaluated with an accuracy of about $\pm$ 10%. Below this range the distance moved is too small for accurate reading and above this range the logarithmic relationship is such that it takes a large change in concentration to induce an increasingly smaller change in distance. Single determinations can be made in the 8-15 μg. and 70-100 μg. ranges with an accuracy of about $\pm$ 20%. The line does not go through the origin but extrapolates to the base line about 5-6 μg. The reason for this is related to the combining properties of the antigen and antibody and the solubility of the complex formed [see (2) for further discussion].

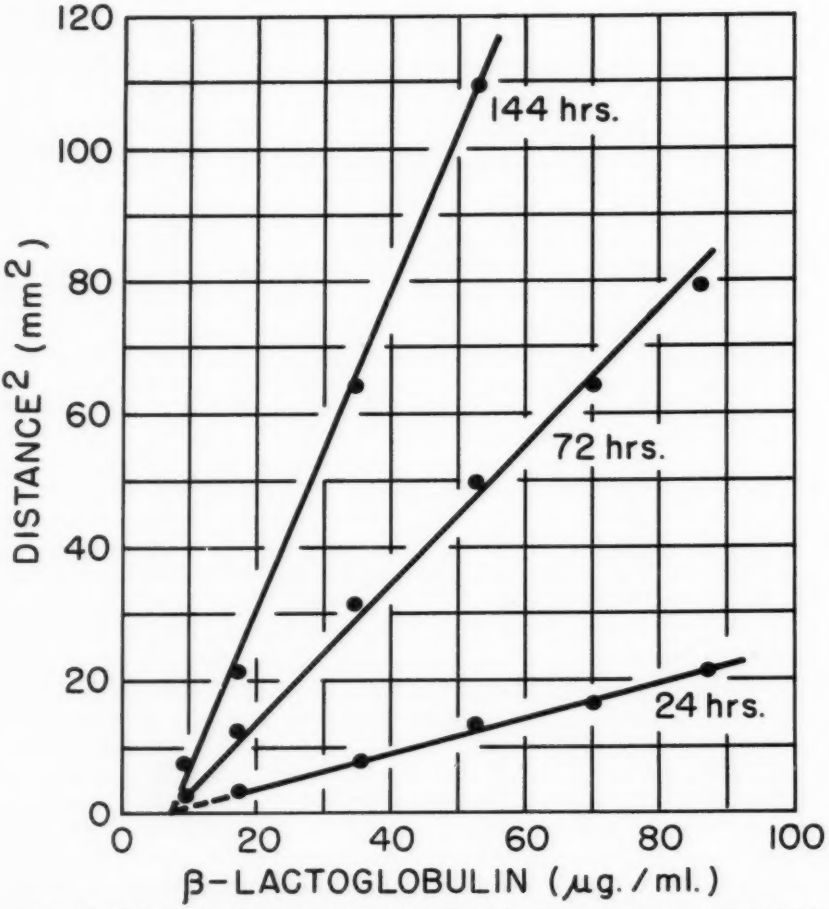

FIG. 2. Relation of the square of the distance (D^2) moved versus the β-lactoglobulin concentration (C). The straight lines tend to slope off above the 80-100-μg. level.

Sample preparation. The sample to be analyzed should be at a pH between 6 and 7 in aqueous solution and contain a level of β-lactoglobulin from 10-80 μg/ml. If the sample must be diluted (such as milk which contains about 2,500 μg/ml), it is preferable to use a buffer that does not contain a high potassium or calcium phosphate level, for it has been observed that such a buffer may produce an interfering ring in the precipitin zone. For a sample already at pH 6-7, saline (0.9% NaCl) can be used as the diluent. For samples outside of this pH range, a dilute buffer should be used such as the pH 6.6 diluting buffer described. If the sample contains only 15-70 μg. per milliliter and ion interference occurs, it can be either diluted down to about 15 μg. per milliliter, or it can be dialyzed against the diluting buffer to lower the level of interfering ions before analysis.

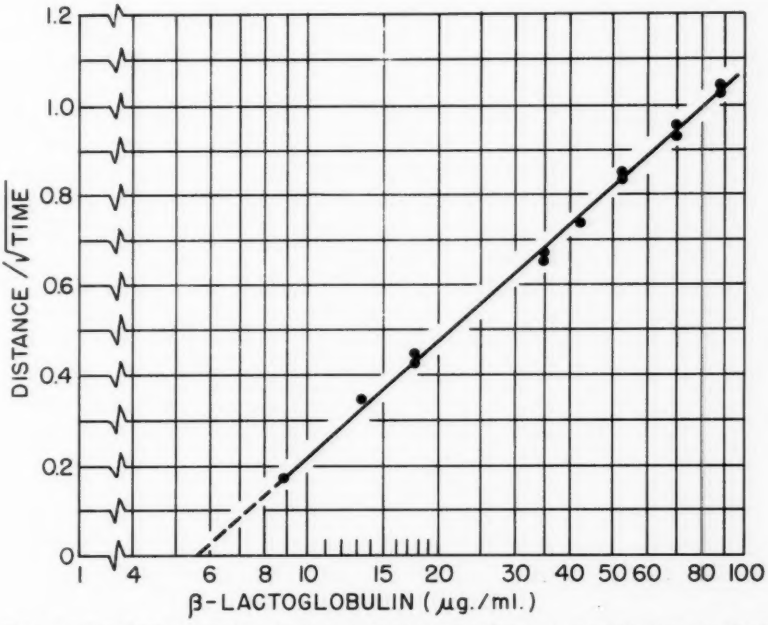

FIG. 3. Standard curve for the immunological procedure. A straight line is obtained either when plotted as shown with $D/\sqrt{T}$ on a linear scale versus C on a logarithmic scale, or it may be plotted as the $D/\sqrt{T}$ versus Log C with both on linear scales.

Milk and other fluid dairy products need only to be diluted 50- or 100-fold with saline (0.9% NaCl) or the pH 6.6 diluting buffer. The β-lactoglobulin in solid products first needs to be solubilized by preparing an aqueous suspension of the sample, using a mechanical blender (or in some cases a glass tissue homogenizer) with the pH 6.6 diluting buffer, then diluting proportionately as needed.

Comments. Although pH is not especially critical around neutrality, the

most consistent results were obtained if the pH of the β-lactoglobulin solution was somewhere between 6 and 7. Temperature is also not a critical factor as long at it remains constant; however, the migration rate decreases with a lower temperature and a different standard curve would have to be prepared for any given temperature. Since antisera prepared against β-lactoglobulin would vary in titer, a new standard curve would have to be prepared for each pooled preparation of antisera [see (2)]. Oudin (14) found it necessary to further clarify the agar to obtain a nonturbid solution. This has not been found necessary in the present study. Some (2) have found it necessary, using larger tubes, to precoat the tubes with a dried agar layer. This has not been necessary using the 2.0-mm. tubes. It might also be mentioned that β-lactoglobulin in very dilute solutions may tend to be somewhat unstable, especially if there are not other proteins present. It is best to make the dilutions needed just before setting up the assay and if storage of the dilute solutions is necessary it should be in the frozen state.

RESULTS

Since β-lactoglobulin is composed of two closely related species now called the A and B forms (3), it was determined whether these forms reacted similarly in the immunological procedure. The β-lactoglobulin used as the antigen in the preparation of the antisera contained both β-lactoglobulin A and B, having been prepared from mixed herd milk obtained from about 250 cows representing five breeds. Since β-lactoglobulin is commonly stored in the laboratory as wet crystals under toluene, but is also available commercially as a dry product through lyophilization, it was determined whether this had an effect on the immunological specificity of the β-lactoglobulin. Results shown in Table 1 indi-

TABLE 1

Immunological reactivity of different preparations of β-lactoglobulin [a]

β-Lactoglobulin preparation	Preparation treatment [b]	Actually present [c]	Amount found [d]
		(μg/ml)	
Mixed [e]	Wet	35	36
		70	72
Mixed [e]	Lyophilized	35	37
		70	70
Pure A [f]	Lyophilized	34	40
		68	66
Pure B [f]	Lyophilized	33	37
		66	71

[a] Each figure is the average of duplicate analyses. Each protein was dissolved in pH 6.6 diluting buffer.

[b] Refers to whether the β-lactoglobulin after several crystallizations was stored in the wet state under toluene or lyophilized and stored in the dry state.

[c] By Kjeldahl analysis (nitrogen × 6.38) of more concentrated solution and then dilution to the level noted.

[d] By immunological assay.

[e] As isolated from mixed herd milk. The wet sample was prepared and stored in this laboratory (10) and from it the lyophilized sample was prepared. A commercial sample of lyophilized β-lactoglobulin also showed similar results.

[f] Pure β-lactoglobulin A and B prepared by Dr. Robert Jenness of the University of Minnesota (from cows producing only A or B β-lactoglobulin).

cate that the antisera to the mixed β-lactoglobulin reacted similarly to the pure A and B forms and that the method of storing the β-lactoglobulin had no effect.

To determine if the presence of other proteins affected the assay procedure, varying levels of β-lactoglobulin from about 8-100 μg. per milliliter were dissolved in various systems, including the regular diluting buffer, casein solutions of up to 3% (acid-prepared casein exhaustively washed and reprecipitated several times and dissolved in the diluting buffer with enough 0.1 *N* NaOH to adjust the pH to about 6.6), and a complex tissue culture medium containing blood serum (see later). In no case were the results any different from those obtained using the regular diluting buffer.

As a further check on the reliability of the method, the immunological analysis procedure was compared with the electrophoretic analysis procedure used previously to evaluate β-lactoglobulin in milk (9, 12, 13, 16). Several milk samples were collected that would be expected to contain varying contents of β-lactoglobulin. The results shown in Table 2 show that there is good agreement between the two procedures.

TABLE 2

β-Lactoglobulin content of skimmilks
Comparison of immunological and electrophoretic procedures

Sample No.	Description	Assay means		
		Electrophoretic [a]	Immunological [b]	
		Average	Average	Range
		(mg/100 ml.)		
1	Holstein, early lactation	280	283	270–300
2	Holstein, mid lactation	200	190	180–200
3	Guernsey, early lactation	210	203	200–210
4	Guernsey, mid lactation	190	187	180–200
5	Jersey, early lactation	280	270	260–280
6	Jersey, late lactation	270	223	200–240
7	Milk 1, heated 72° for 30 min.[c]	160	180	170–190
8	Milk 1, heated 100° for 30 min.[d]	0	0	0
9	Colostrum, 2nd milking	420	390	370–400
10	Colostrum, 3rd milking	340	450	400–500

[a] This method is estimated to have about a ± 15% accuracy (see text). Note in comparing these results to those recorded previously (12, 16) that correction has been made for the proteose-peptone components (3) in the β-lactoglobulin area of the electrophoretic pattern.

[b] Each sample of milk was diluted 50-fold with pH 6.6 diluting buffer. Each result is the average of separate readings at three times (24, 48, and 72 hr.). The ranges show how these readings varied.

[c] This heat treatment should denature about 40% of the β-lactoglobulin as shown by its precipitation with casein [see (13)]; the electrophoretic assay indicates 43% and the immunological assay 36%.

[d] This heat treatment should denature 100% of the β-lactoglobulin (13).

The method was further applied for the analysis of the β-lactoglobulin content of various milks and milk products. Results shown in Table 3 indicate that the β-lactoglobulin content of these products was readily assayed and these results suggest that any product from which β-lactoglobulin can be extracted and put into aqueous solution can be evaluated readily. Furthermore, the presence of emulsified fat such as is present in whole milk does not interfere in the test.

TABLE 3

β-Lactoglobulin content of various milks and milk products as determined by the immunological procedure

Sample No.	Description [a]	β-Lactoglobulin content [b]	
		(mg/100 ml.)	*(mg/100 g.)*
1	Ayrshire milk, whole, raw	230	.....
2	Ayrshire milk, skim, raw	270	.....
3	Ayrshire milk, whole, pasteurized [c]	260	.....
4	Brown Swiss milk, whole, raw	300	.....
5	Brown Swiss milk, skim, raw	320	.....
6	Brown Swiss milk, pasteurized [c]	300	.....
7	Milk, 3.5% B.F.	240	.....
8	Chocolate-flavored milk, 3.5% B.F.	220	.....
9	High nonfat solids milk, 2.0% B.F.	240	.....
10	Nonfat dry milk solids (instant)	.....	1,700
11	Process cheese	.....	130
12	Cheddar Cheese (short-aged)	.....	100
13	Blue cheese	.....	0

[a] Samples 1-3 and 4-5 represent single milk samples from two cows. Samples 7-13 were purchased on the open market.

[b] Samples 1-9 were diluted 50-fold with pH 6.6 diluting buffer for the assay. Samples 10-13 were reconstituted at 10 g. per 100 ml. of final volume in pH 6.6 diluting buffer. A Waring Blendor was used for Samples 11-13. The blended samples were filtered through cotton. Sample 10 was further diluted 50-fold and Samples 11-13 were diluted tenfold with the diluting buffer. The values shown are for the β-lactoglobulin content in the original product.

[c] 61.7° C. for 30 min.

The level found in the skimmilk tends to be slightly higher than that found in the whole milk, probably due to the diluting effect of the fat in the whole milk.

Due to the high β-lactoglobulin content of milk, it is extremely unlikely that any product derived from or containing milk would contain less than 10 μg/ml of β-lactoglobulin. It is conceivable, however, that there may be occasions when it might be desirable to evaluate a concentration of β-lactoglobulin below this level. This situation was encountered when the synthesis of β-lactoglobulin was being studied in in vitro cell cultures derived from the mammary gland and containing a nutrient medium (5, 6). Thus, a procedure was derived to determine in this nutrient medium levels of β-lactoglobulin well below 10 μg/ml. This procedure will be described to indicate how the general method can be adapted to evaluate these low levels. It will be apparent, however, that the procedure described might not be applicable to another system without first working out the quantitative relationships involved.

The nutrient medium used to maintain mammary secretory cells in culture was composed of 20% bovine serum and 40% each of two commercial synthetic media, 199 medium and Eagles' basal medium. This composited medium contains glucose, various salts, amino acids, vitamins, purine and pyrimidine bases, nucleotides, and other metabolites contributed by the two synthetic media, plus a serum protein level of about 1.5% in addition to the multitude of materials present in blood serum [see (5, 6) for further information]. Varying levels of β-lactoglobulin from about 10 μg/ml to 80 μg/ml were prepared in the medium and run in the regular assay procedure. The results were the same as

obtained using β-lactoglobulin dissolved in the regular diluting buffer. Thus, the materials in the complex medium did not interfere in the regular assay procedure.

Various levels of β-lactoglobulin in the culture medium were then subjected to the following concentration procedure: Two milliliters of medium were placed in a graduated centrifuge tube and freeze-dried under vacuum (lyophilized). Contents of the tubes were dissolved in diluting buffer and brought up to an appropriate volume, i.e., 1.0 ml. for a twofold, 0.5 ml. for a fourfold, and 0.25 ml. for an eightfold concentration (an eightfold concentrated solution would now contain about 12% of blood serum proteins). The concentrated solutions were then run in the regular assay procedure.

Results from all of the concentration levels indicated that there was a twofold increase in the apparent β-lactoglobulin level above that which should have been due to the concentration procedure. For example, a solution containing 1 μg/ml when concentrated eightfold from 2.0 to 0.25 ml. showed an analysis level of 16 μg/ml instead of the predicted 8 μg/ml. Furthermore, a solution containing 17.5 μg/ml, when assayed before lyophilization, showed a level of 17.5 μg/ml, but when lyophilized and then made back to the same volume without any concentration now assayed at 35.0 μg/ml. Further investigation indicated that this apparent concentration factor of two above the predicted level occurred only with the dilute solutions (0 up to about 50 μg/ml) of β-lactoglobulin in the medium and was due to the process of lyophilization; merely freezing and thawing did not affect a change in the apparent level. Since there is no need to concentrate the solution if the β-lactoglobulin level is above 10 to 15 μg/ml, the doubling occurs in a predictable manner when the concentration procedure is necessary. In order to check if the doubling occurred with the β-lactoglobulin synthesized in the cultures as opposed to that added to the medium, media removed from the culture flasks and containing more than 10 μg/ml were analyzed before and after lyophilization, with and without actual concentration. Results obtained were similar to those found with the added β-lactoglobulin, in that the assay results after lyophilization were double the values found with the assay procedure before lyophilization.

The doubling in the apparent immunological reactivity of β-lactoglobulin when lyophilized in very dilute solutions helps to increase the sensitivity of the assay. Thus, with the concentration procedure it is possible to determine a level of β-lactoglobulin down to 0.6 μg/ml which, when concentrated eightfold by lyophilization, gives a 16-fold increase in antigenicity to an effective level of 9.6 μg/ml, which can be assayed in the regular procedure. Since it is not known how wide-spread this doubling effect may be in systems other than the one described using tissue culture medium, it is apparent that if the occasion arose where it was desirable to evaluate levels of β-lactoglobulin below 10 μg/ml in some other system, the stoichiometry would have to undergo scrutiny similar to that applied here.

The effect of lyophilization in doubling the apparent immunological titer when lyophilized in dilute solution prompted the question of whether other

β-lactoglobulin preparations when diluted, especially those that had been previously lyophilized, would also show this doubling phenomenon. Samples of β-lactoglobulin stored both as wet crystals under toluene and as a dry lyophilized powder were dissolved in the regular tissue culture medium, then lyophilized. Similar results were obtained from both the wet and the lyophilized samples, i.e., both showed the apparent doubling in the immunological titer upon lyophilization, but only as dilute solutions. These results are consistent with what might be expected. When β-lactoglobulin is prepared for storage in the laboratory, its concentration at lyophilization is several hundred times more concentrated than the level at which the process appears to effect a doubling of the immunological titer.

The reason for the twofold increase in the apparent β-lactoglobulin level is not known at the present time. The increase appears to be a function of the physical state of the β-lactoglobulin molecule which, when lyophilized in a very dilute state in the tissue culture medium, changes in form so as to double the apparent number of immunologically reactive sites available. Further studies are in progress to elucidate this phenomenon.

DISCUSSION

The use of an immunological procedure to determine β-lactoglobulin was previously reported by Deutsch (4), who prepared antisera to β-lactoglobulin and used it in an agglutination test assay. In these studies a pronounced heterogeneity was found in the preparations of crystalline β-lactoglobulin used and evidence was presented for their contamination with at least one and possibly more serum protein components. Using the agglutination procedure with a sample of milk serum, Deutsch (4) found that the immunological assay procedure indicated a significantly higher level than that found by electrophoretic analysis. It may be for this reason that immunological methods to determine β-lactoglobulin have received little attention in recent years.

In the present investigation it has been observed that the crystalline β-lactoglobulin used as the antigen to produce an antibody response must be scrupulously purified by many successive recrystallizations. β-Lactoglobulin appears to be a weak antigen, compared to many other proteins. Even though physical methods such as electrophoresis and ultracentrifugation may indicate the β-lactoglobulin to be pure, if it is not sufficiently purified minute quantities of other proteins present [notably the eu- and pseudoglobulin fractions and serum albumin present in milk but originally derived from blood (9)] will elicit a significant immunological response to them. These observations may well explain the results that Deutsch (4) encountered. Whereas an agglutination test is based on the measurement of the total amount of agglutinated material present, the agar-diffusion method used in the present investigation rapidly indicates heterogeneity in the antigen-antisera system. If contaminating proteins are present in the antigen eliciting an antibody response which appears in the antisera, instead of a single diffusion zone appearing, a zone with different diffusion rates will appear for each impurity. Since a single zone resulted, it indicates that the antigen elicited a single antibody response. As a further confirmation of this,

the antisera was run in precipitin ring tests (10) against whole bovine serum and all of its major protein fractions (serum albumin, α-, β-, and γ-globulin mixtures) and all of the other major milk protein fractions (α-, β-, and γ-casein, eu- and pseudoglobulin fractions, and α-lactalbumin). There was no perceivable response for any of the blood materials or the casein proteins at any of the various dilutions tried. The small response to the eu- and pseudoglobulin fractions and α-lactalbumin could be accounted for by the small amount of β-lactoglobulin contaminating these preparations.

Results in Table 1 indicate that immunologically β-lactoglobulin A and B are indistinguishable. This indicates that the amino acid residues which differ between the two forms (15) are not concerned with the immunologically reactive sites and, hence, the method is equally applicable to the milk from all cows.

Results in Table 2 show good agreement between the immunological and the electrophoretic procedure and indicate that both methods are determining essentially the same thing. Since the immunological method is based on a discrete property of the β-lactoglobulin molecule, rather than a physical property which other molecules could also possess, the immunological procedure is probably more accurate than the electrophoretic procedure,[4] and much easier to run. In the total expended time required to conduct one electrophoretic analysis requiring expensive instrumentation and trained technical help, over 100 immunological assays could be conducted using essentially minimum trained help and no more instrumentation than a source of constant temperature and a pair of calipers.

Although extended observations were not conducted, results in Table 2 also indicate that there is a close similarity between the denaturability of β-lactoglobulin with heat as determined by both the electrophoretic and the immunological procedure. In both cases, it was indicated that the β-lactoglobulin content of milk heated at 100° C. (30 min.) was completely denatured, and a heat treatment of 72° C. (30 min.) denatured about 40% (13).

It is interesting to note from results in Table 3 the high level of β-lactoglobulin present in the sample of instant nonfat dry milk solids tested. This indicates that 1.7% of the product was undenatured β-lactoglobulin. Of the cheese samples analyzed, the process cheese sample contained the most β-lactoglobulin and none could be found in the sample of the blue cheese. The assay results appear to correlate with the age of the cheese and, thus, the degree of proteolysis of β-lactoglobulin that would be expected to have occurred in the samples.

[4] The intricacies of electrophoretic procedure and the inherent shortcomings in the isolation procedure from milk and in the electrophoretic analysis procedure make it difficult to ascribe a statistical evaluation of the accuracy range for the method. From many such analyses conducted (8, 12, 13, 16) an accuracy within 10% is ascribed as a fair estimate of variability in the method. Since milk samples contain varying amounts of other components with mobilities similar to β-lactoglobulin, perhaps another 10% in variability could be ascribed, especially in milk samples near the extremes in the lactation period, where the presence of significant amounts of some of the blood proteins complicate the situation (8, 12). Thus, over-all a variability of plus or minus 15 or 20% in the true level of β-lactoglobulin in a sample of milk is probably the accuracy range of the electrophoretic analysis procedure.

Aschaffenburg and Drewry (1) have published a chemical method to evaluate β-lactoglobulin in milk based on salt fractionation procedures and nitrogen analyses. Although a direct comparison with the electrophoretic procedure was not made with actual analyses, the results of the chemical method were compared with the results of previous electrophoretic investigations in this laboratory (9). The close comparison suggested strongly that the method was actually determining β-lactoglobulin. This chemical method is undoubtedly easier to conduct than the electrophoretic procedure, but would not be applicable to milk products containing either low levels of β-lactoglobulin or in the presence of other proteins that possess similar precipitability properties to β-lactoglobulin.

Yaguchi *et al.* (18) have recently published work describing methods to separate individual proteins from milk on anion-exchange cellulose columns. This approach shows great promise as a means both of identifying and of isolating significant quantities of the individual milk proteins. The results of further research may well develop this procedure into a method for quantitating individual proteins in milk that may be more applicable than the electrophoresis procedure. It is unlikely, however, that the procedure could be developed into a simple method for the routine determination of an individual milk protein, especially if it is present in low concentration and/or in a variable system containing proteins other than those of milk.

One of the major advantages of the procedure described in this paper is that the determination of β-lactoglobulin is based on the immunological specificity of the β-lactoglobulin molecule. However, a property that could render difficulty in such an immunological procedure is the presence of another protein with similar immunological characteristics so as to cause a cross reaction with the antisera to the β-lactoglobulin. Such a possibility is extremely remote. No cross reaction has been observed with the other milk proteins or with any protein in whole blood serum or in a variety of bovine tissue homogenates prepared from several different organs. If such a cross reaction did exist in a solution under assay, it should give an additional zone in the agar diffusion tubes; none has been observed.

It is apparent that the principles of the immunological procedure described in this paper should be applicable to the determination of several other proteins present in milk. For several of these proteins the electrophoretic procedure is still the only method that has been described to quantitate their presence in milk. At the present time the development of such a method to evaluate two or three more of the proteins in milk looks promising. These studies are continuing and will be reported in subsequent communications.

This method to evaluate the β-lactoglobulin has many advantages in simplicity and sensitivity over any method previously described and is applicable to situations where no other method has been available. The sensitivity of the procedure in evaluating less than 1 μg. of β-lactoglobulin per milliliter has made it possible to demonstrate and to follow quantitatively the synthesis of β-lactoglobulin in in vitro cell cultures derived from the mammary gland, and thus has been a valuable tool in this area of studying the biochemistry of milk forma-

tion (6). The ease with which the assay may be conducted on milk and milk products, using essentially simple equipment and minimum trained personnel, indicates that this assay could be conducted as easily as the Babcock test for fat or the titration test for acidity if the occasion so warranted. In recent years there has been an increasing need for methods to evaluate certain individual milk proteins that may be causing problems associated with variability between different milk sources. The intricacies of the available methods to determine these individual proteins seemingly have discouraged more general work in this area. The development of more simplified procedures to determine additional individual milk proteins should help efforts in this direction.

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HEREDITARY VARIATION IN CONCEPTION RATE OF HOLSTEIN-FRIESIAN CATTLE [1]

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SUMMARY

Variation in conception rates (based on birth of a live calf from first insemination to a high-fertility service sire) of 1,406 Holstein females was studied at one or two service periods (1,824 records) in 41 artificially inseminated herds. Fertility did not vary significantly between herds.

Conception rates ranged from 43 to 94% in 27 paternal half-sib groups of heifers ($P < 0.05$ on an average of 23.4 heifers per half-sib group) and from 33 to 91% in 36 paternal half-sib groups of parous cows ($P = 0.05$ on an average of 22.7 parous cows per half-sib group).

The heritability of conception rate was estimated from intrasire correlations of paternal half-sib groups to be approximately 8.5%.

A comparison of the records of daughters at both service periods from 22 sires showed a trend toward an interaction between sire and parity of daughters ($P \cong 0.07$).

The value of selection in improving conception rate is discussed.

Previous investigations have shown little evidence for genetic variability in conception rate in dairy cattle.

Dunbar and Henderson (3) considered nonreturn to first service by 180 days as an indication of conception in production-tested Holstein cows. Heritability estimated from the variance due to sires was near zero.

At the Danish progeny testing stations, heritability estimates for conception rate at first and second services, in heifers of three breeds, bracketed zero (6).

Among cows of an experimental Holstein herd, Mares *et al.* (4) found highly significant differences, depending on the ancestral sire-line to which the cows belonged, and also significant differences between the sire-lines in the effects of inbreeding and line-crossing on conception rate at first service. Only sire-line differences in the effects of inbreeding and line-crossing on the embryo, as a factor in conception rate, were significant for heifers in the same herd.

The following study of farm herds was undertaken to analyze the variation in conception rates of Holstein females from different sires, when bred artificially to high-fertility Holstein bulls.

MATERIALS

Data were tabulated from the barn breeding records and calf record books of 41 Holstein herds in which cows were inseminated artificially by Badger Breeders Cooperative, Shawano, Wisconsin.

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Records were taken from a period in which no major changes were made in the techniques of semen processing and handling (1953 to 1958). For each year, the Holstein sires in the Badger Breeders stud were ranked according to 60-90 day nonreturn rates (based on 100 or more services per month of service). The upper two-thirds of these bulls in each year were considered high-fertility sires; their nonreturn rates ranged from 65.4 to 78.2%. The stud average nonreturn rates for Holsteins during these years ranged from 67.0 to 71.8%.

Cows bred to a highly fertile service sire were considered to have conceived if the first insemination resulted in the birth of a live calf. These breedings were coded 1; breedings resulting in failure to produce a live calf were coded 0.

First-service records from one or two service periods were collected for each individual cow when bred to a high-fertility bull: her heifer record and/or her earliest available parous record. All cows included were daughters of bulls in current or former service in the Badger Breeders stud.

RESULTS AND DISCUSSION

A total of 1,824 records, from 41 farms, of 1,406 cows bred to the selected highly fertile bulls showed a mean of 66.9% producing live calves to first service. The mean 60-90 day nonreturn rate of the selected bulls was 72.7%. The diffrence between these values estimates that 5.8% of cows bred to highly fertile bulls, which do not return to service, actually do not produce live calves. This is comparable to the reports of Barrett *et al.* (2) and Adler (1), that 60-90 day nonreturn rates were 5.5 percentage points higher than the percentages of pregnancies diagnosed by rectal palpation.

Differences in conception rates among farms were tested by Chi-square (1,824 records, $P \cong 0.07$).

Arbitrarily, only progeny groups consisting of at least ten daughters per bull were used to study the effect of sires on the reproductive performance of their daughters. Thirty-six such groups furnishing a total of 817 records were available from the data on parous cows, and 27 groups furnishing 633 records were available from the data on heifers (324 of the heifer records were on the same animals as furnished parous records). Inasmuch as the herd effect was relatively small, and the number of herds per progeny group averaged approximately 15, no adjustment for herd differences was made.

The conception rates of paternal half-sib groups varied from 43 to 94% ($P < 0.05$) at the heifer service period, $\bar{x} = 67.3\%$, and from 33 to 91% ($P = 0.05$) at the later (parous) service period, $\bar{x} = 68.3\%$ (Table 1).

Intra-sire correlations obtained from analysis of variance (Table 2) were 0.023 for heifers and 0.019 for parous cows. Since the expectation of these correlations is $0.25 \frac{\sigma^2 G}{\sigma^2 T}$, where $\sigma^2 G$ is the additive genetic variance and $\sigma^2 T$ is the total phenotypic variance, they are multiplied by four to estimate heritability. Thus, the heritability estimates were 0.09 and 0.08 for heifers and parous cows, respectively. These values may differ from the estimate of zero made by Dunbar and Henderson (3), who studied only production-tested cows, and based

TABLE 1

Conception rates of sire progeny groups

Bull	Heifer records			Cow records			Total No. of daughter records	Daughters average conc. rate (%)
	No.	No. of farms	Conception (%)	No.	No. of farms	Conception (%)		
1	18	15	94.4	18	12	66.7	36	80.6
2	15	11	66.7	21	13	90.5	36	78.6
3	25	18	76.0	26	18	80.8	51	78.4
4	31	20	90.3	53	23	66.0	84	78.2
5	20	14	80.0	32	20	75.0	52	77.5
6	15	11	73.3	21	17	76.2	36	74.8
7	17	14	76.5	21	16	71.4	38	74.0
8	24	16	70.8	30	18	76.7	54	73.8
9	14	8	85.7	28	18	60.7	42	73.2
10	15	11	80.0	15	12	60.0	30	70.0
11	41	21	70.7	21	14	61.9	62	66.3
12	43	22	67.4	49	23	63.3	92	65.4
13	80	33	62.5	72	29	66.7	152	64.6
14	32	24	65.6	24	19	62.5	56	64.0
15	27	19	51.9	27	21	74.1	54	63.0
16	23	15	65.2	22	16	59.1	45	62.2
17	20	15	55.0	31	18	67.7	51	61.4
18	16	12	56.2	20	14	60.0	36	58.1
19	23	15	47.8	25	18	68.0	48	57.9
20	25	12	68.0	16	12	43.8	41	55.9
21	21	15	42.9	19	14	63.2	40	53.0
22	23	17	52.2	10	8	50.0	33	51.1
23	10	9	80.0					
24	14	12	71.4					
25	15	12	66.7					
26	14	11	64.3					
27	12	10	58.3					
28				10	7	90.0		
29				19	10	89.5		
30				27	16	88.9		
31				15	11	86.7		
32				10	8	80.0		
33				14	10	78.6		
34				19	10	73.7		
35				17	11	70.6		
36				10	9	70.0		
37				11	8	63.6		
38				10	9	60.0		
39				23	14	52.2		
40				16	13	50.0		
41				15	11	33.3		
Σ's	633	412		817	520		1,169	
$\bar{x}$'s	23.44	15.26	67.3[a]	22.69	14.44	68.3[a]	53.1	67.4[a]

[a] Unweighted means of all half-sib groups containing ten or more individuals.

TABLE 2

Analysis of variance

Source	Heifer service period			Parous service period		
	d.f.	Mean square	Variance	d.f.	Mean square	Variance
Total	632			816		
Between sires	26	.3358[a]	.0052	35	.3057[a]	.0041
Within sires	606	.2155	.2155	781	.2128	.2128

[a] $P \leqslant 0.05$.

conception on nonreturn rates. They did not consider variation in service-sire fertility, and heifers that never conceived were automatically excluded. The present estimate for parous cows, however, also excludes such heifers.

Twenty-two bulls had progeny groups in both parity classes. A Chi-square method described by Rao (5) was used to test the effects of parity of daughter, and the interaction between sire and parity of daughter, on the conception rate. Differences between half-sister groups were again significant ($P < 0.05$). Mean conception rates at the two service periods were similar (68.1 and 66.6; $P > 0.05$). There was a trend toward an interaction between the sire and the parity of his daughters ($P \cong 0.07$), the progeny of some sires excelling in conception rate as heifers, whereas the daughters of other sires had higher conception rates as parous cows.

Since this study indicates that the heritability (additive genetic variance) of conception rate in Holstein cows bred artificially to highly fertile bulls is about 8.5%, the mean conception rates of paternal half-sib groups may provide criteria for selecting for higher conception rates.

Selecting herd replacements. The expected difference in conception rate between future daughters of a selected group of sires and future daughters of all sires can be estimated as follows:

$$\text{Expected gain} = \frac{nh^2}{4[1 + (n-1)r]} \times sd \times s,$$

where, n is the number of daughters per bull,
h^2 is the estimated heritability of conception rate,
r is the correlation among paternal half-sibs,
sd is the selection differential of the selected group,
and, s is the standard deviation of the paternal half-sib groups.

The improvements in conception rate that the breeder could expect from selection were calculated for several sample sizes and selection intensities, and are shown in Table 3.

Selecting A.I. sires. To illustrate possible gains from selecting bulls on the basis of the performance of their paternal half-sisters, the data on 22 sires with progeny groups at both service periods in the present study have been considered. The mean conception rates of these daughter groups ranged from 51.1 to 80.6%. The mean fertility was 75.0% for the upper 11 groups and 59.7% for the lower 11 groups. Assuming 30 daughters per sire ($\bar{x} = 53$ daughter

TABLE 3

Expected improvement in conception rate by selection of daughters of bulls whose previous daughters showed a high conception rate

Selecting daughters from:	Increase in conception rate above daughters of all sires		
	No. of daughters per sire group		
	10	20	30
Upper 25% of sires	0.036	0.047	0.054
Upper 50% of sires	0.023	0.030	0.034
Upper 70% of sires	0.014	0.019	0.021
Upper 80% of sires	0.010	0.013	0.015
Upper 90% of sires	0.006	0.008	0.009

records/sire for the above 22 sires), the regression of the breeding value of a sire on the performance of his daughters is 0.79. When this regression is multiplied by the difference between the high and low group means (15.3%), an estimate of 12.1% difference in the breeding values of the two groups of sires is obtained. Since a bull is expected to transmit approximately one-half of his breeding value to his sons, the paternal half-brothers of the high daughter groups should have a mean breeding value 6.0% higher than the paternal half-brothers of the low daughter groups.

TABLE 4

Expected differences in conception rates between future daughters of two proven sires

Difference in conception rate of previous daughters	Expected difference in conception rate of future daughters	
	No. of previous daughters	
	10	20
(%)		
5	0.008	0.015
10	0.018	0.030
15	0.027	0.045
20	0.036	0.061

Sire-proving programs could be modified to obtain progeny information on fertility. Thus, differences in breeding value might become important in selecting bulls to bring into a stud for extensive use. The expected differences in future daughters' conception rates between two bulls whose previous daughters' mean conception rates differed by 5 to 20% are shown in Table 4. These values were calculated by multiplying the differences between the bulls' previous daughters by the regression of the performance of future daughters on the performance of previous daughters.

Elimination from the stud of bulls whose daughters showed poor conception rates should result in improvements similar to those from daughter selection shown in Table 3. The extent to which previous daughters of the discarded sires were retained in the population would affect the gains in conception rate, so the exact value of a bull-culling program is difficult to estimate.

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ESTIMATION OF MISSING MONTHLY TEST-DAY RECORDS

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SUMMARY

Three methods, within herd regression, regression ignoring herd effects, and a ratio factor method, of estimating a missing monthly test-day record from preceding or succeeding known test-day records are compared. The intra-herd regression method is considered to be better than regression ignoring herd effects or the ratio factor method. The regression procedure ignoring herd effects, however, is generally less than 10% less efficient than the intra-herd method and is recommended for use, since population averages are easier to obtain and to use than are the herd averages required for the intra-herd method. Factors are presented for estimating a missing monthly test-day record by the three methods when (1) the preceding monthly record is known, (2) the succeeding monthly record is known, and (3) both preceding and succeeding records are known.

The favorable acceptance of DHIA central processing of dairy records has brought with it many problems. One of these is what to do about missing test-day records, which occur frequently, O'Bleness (3). Gross comparison factors obtained from ratios of average monthly test-day records for predicting missing monthly test-day records were presented by Kendrick (2) in 1940. Since that time little attention has apparently been given to this problem. The purpose of this study was to develop factors to predict a missing monthly test-day milk record when (1) the previous monthly test-day record is known, (2) the succeeding monthly record is known, and (3) both the preceding and succeeding monthly records are known by regression ignoring herd effects (total regression), intra-herd regression, and the ratio of adjacent monthly test-day averages.

DATA

The DHIA milk records used in this study were from 9,036 Holstein cows in 374 New York herds. Ten monthly test-day records were available for each cow's lactation, beginning with the first month of lactation and ending with the tenth month. The sum of the first ten test-day records was defined to be a complete lactation yield. Actual 305-day yield can be estimated by multiplication of this sum by 30.5, the number of days in the average DHIA month. The monthly records were adjusted to a common age at calving (72-77 mo.) and season of calving (December-March) by the use of ratio factors described by VanVleck and Henderson (5).

METHODS OF ESTIMATION

Suppose that the test-day record for the i^{th} month of lactation was not reported and that the test-day record for the adjacent j^{th} month is known. Then, a procedure is required for estimating the i^{th} record from the j^{th}. Three methods

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will be proposed and discussed. It will be assumed that the records have been adjusted for all external effects, such as age at calving and season of calving, except herd effects.

Method 1. The record of the i^{th} month can be estimated from the intra-herd regression of the i^{th} monthly record on the j^{th} monthly record. The prediction equation is

$$y_{i1} = \mu_i' + B_j(x_j - \mu_j') \; ; \; j = i-1 \text{ or } i+1 ;$$

where y_{i1} is the predicted monthly record,
μ_i' is the herd average for the i^{th} month of lactation,
x_j is the known monthly record,
B_j is the intra-herd regression factor associated with x_j, and
μ_j' is the herd average for the j^{th} month of lactation.

Method 2. The record of the i^{th} month can be estimated from the total regression (ignoring herd effects) of the i^{th} monthly record on the j^{th} monthly record. The prediction equation is

$$y_{i2} = \mu_i + b_j(x_j - \mu_j) \text{ or,}$$
$$y_{i2} = a_i + b_j(X_j) \; ; \; a_i = \mu_i - b_j\mu_j ; \; j = i-1 \text{ or } i+1 ;$$

where y_{i2} is the predicted monthly record,
μ_i is the population average for the i^{th} month,
x_j is the known monthly record,
b_j is the total regression factor associated with x_j, and
μ_j is the population average for the j^{th} month of lactation.

Method 3. The ratio factor method ignoring herd effects of estimating the i^{th} monthly test-day record from the j^{th} monthly record is

$$y_{i3} = \frac{\mu_i}{\mu_j} x_j = \mu_i + \frac{\mu_i}{\mu_j} (x_j - \mu_j) \; ; \; j = i-1 \text{ or } i+1 ;$$

where y_{i3} is the predicted monthly record,
μ_i is the population average for the i^{th} month,
μ_j is the population average for the j^{th} month, and
x_j is the known monthly record.

Bias. Under the condition that the records are corrected for all effects (age, season, year, etc.), except herd effects, Method 1 will give the best linear unbiased estimates of missing monthly test-day records. There are, however, considerations which make this method undesirable. Therefore, the biases of the other two procedures are of interest, so that these biases can be balanced against the objectionable features of Method 1, to determine which procedure to use in the normal operation of a records processing center. Assuming Method 1 is best, the bias of another procedure is the difference between Method 1 and the alternative method. Then, the bias of Method 2 relative to Method 1 is

$$\begin{aligned} d_{12} &= y_{i1} - y_{i2} \\ &= (\mu_i' - \mu_i) + (b_j\mu_j - B_j\mu_j') + (B_j - b_j)x_j. \end{aligned}$$

The bias of Method 3 relative to Method 1 similarly is

$$d_{13} = \mu_i' - B_j\mu_j' + (B_j - \frac{\mu_i}{\mu_j})x_j.$$

The bias of Method 3 relative to Method 2 as shown by Harvey (1) is

$$d_{23} = (b_j - \frac{\mu_i}{\mu_j})\ (x_j - \mu_j).$$

Inspection of d_{12} reveals if the total and intra-herd regressions are equal, $b_j = B_j = c_j$, then the bias is strictly accounted for by the differences of the herd averages from the population averages. In fact, in that case $d_{12} = (\mu_i' - \mu_i) + c_j(\mu_j - \mu_j')$. Actually, if c_j is near unity then the bracketed components of the bias tend to balance each other. In practice, it is doubtful whether the bias is corrected by this compensating force. The difference between Methods 1 and 3, d_{13}, also exhibits this compensating influence. If $B_j = \frac{\mu_i}{\mu_j}$, then the constant bias is $\mu_i' - \frac{\mu_i\mu_j'}{\mu_j}$, which is a function of the population and herd averages. This bias would approach zero as the herd averages approach the population averages and, conversely, the bias would increase with an increased disparity between the herd and population averages. The bias of Method 3 relative to Method 2 approaches zero as the total regression coefficient, b_j, approaches the ratio of monthly averages, $\frac{\mu_i}{\mu_j}$.

The corresponding estimates of the missing monthly test-day records when both the preceding and succeeding records are known are

$$y_{i1} = \mu_i' + B_{i-1}(x_{i-1} - \mu_{i-1}') + B_{i+1}(x_{i+1} - \mu_{i+1}');$$
$$y_{i2} = \mu_i + b_{i-1}(x_{i-1} - \mu_{i-1}) + b_{i+1}(x_{i+1} - \mu_{i+1})\text{; and}$$
$$y_{i3} = \frac{\mu_i}{\mu_{i-1} + \mu_{i+1}}\ (x_{i-1} + x_{i+1}).$$

The biases similarly are

$$d_{12} = \mu_i' - \mu_i + (b_{i-1}\mu_{i-1} - B_{i-1}\mu_{i-1}') + (b_{i+1}\mu_{i+1} - B_{i+1}\mu_{i+1}') + (B_{i-1} - b_{i-1})x_{i-1} + (B_{i+1} - b_{i+1})x_{i+1}$$

$$d_{13} = \mu_i' - B_{i-1}\mu_{i-1}' - B_{i+1}\mu_{i+1}' + (B_{i-1} - \left(B_{i-1} - \frac{\mu_i}{\mu_{i-1} + \mu_{i+1}}\right)x_{i-1} + \left(B_{i+1} - \frac{\mu_i}{\mu_{i-1} + \mu_{i+1}}\right)x_{i+1}, \text{ and}$$

$$d_{23} = \left(b_{i-1} - \frac{\mu_i}{\mu_{i-1} + \mu_{i+1}}\right)(x_{i-1} - \mu_{i-1}) + \left(b_{i+1} - \frac{\mu_i}{\mu_{i-1} + \mu_{i+1}}\right)(x_{i+1} - \mu_{i+1}).$$

These biases behave similarly to those described previously, when only one adjacent test-day record is known, but are slightly more complex.

FACTORS

Ratio factors for predicting a missing monthly test-day record are shown in Table 1. The values presented indicate that an average factor for all months

TABLE 1

Ratio factors for predicting a missing monthly test-day record when (1) the preceding test-day record is known, (2) the succeeding test-day record is known, and (3) both the preceding and succeeding test-day records are known

	Monthly test-day record to be estimated									
	1	2	3	4	5	6	7	8	9	10
(1)[a]	....	1.05	.95	.91	.90	.89	.85	.80	.67	.62
(2)[b]	.95	1.05	1.10	1.11	1.13	1.18	1.24	1.50	1.60	....
(3)[c]	....	.52	.51	.50	.50	.51	.50	.52	.47	....

[a] To estimate the test-day record for the i^{th} month, multiply the factor under month i by the test-day record of month $i-1$.

[b] To estimate the test-day record for the i^{th} month, multiply the factor under month i by the test-day record of month $i+1$.

[c] To estimate the test-day record for the i^{th} month, multiply the factor under month i by the sum of the test-day records of months $i-1$ and $i+1$.

is not appropriate, since the factors are dissimilar, especially for the extreme end months of lactation. When the preceding record is known, the factors decrease as expected with stage of lactation, but not at a constant rate. Conversely, when the succeeding record is known, the factors increase with the length of the lactation. The factors change little with stage of lactation when both adjacent monthly records are known. The usual procedure of using the average of the two adjacent records to predict the missing record is evidently a close approximation, since the factors are all near 0.5. The ratio factors presented here do not agree with those reported earlier by Kendrick (2), the average lactation curve being much flatter in his study.

Total and intra-herd regression factors are given in Table 2 for estimating a missing monthly record when either the preceding or succeeding record is

TABLE 2

Total and intra-herd regression factors for estimating a missing monthly test-day record when (1) the preceding test-day record is known and (2) the succeeding test-day record is known [a]

Test-day record	(1)			Test-day record	(2)		
	a_i	b_{i-1}	B_{i-1}		a_i	b_{i+1}	B_{i+1}
2 from 1	11.8	.84	.70	1 from 2	20.6	.61	.52
3 from 2	5.5	.86	.80	2 from 3	10.9	.86	.80
4 from 3	5.6	.81	.75	3 from 4	7.8	.95	.90
5 from 4	5.9	.79	.73	4 from 5	8.7	.92	.85
6 from 5	2.7	.83	.79	5 from 6	8.1	.93	.85
7 from 6	1.2	.82	.79	6 from 7	8.8	.93	.86
8 from 7	− 0.6	.82	.80	7 from 8	12.0	.82	.75
9 from 8	− 4.0	.81	.81	8 from 9	13.2	.80	.74
10 from 9	− 3.9	.83	.83	9 from 10	10.5	.71	.68

[a] a_i is the intercept of the prediction equation $y_i = a_i + b_j x_j$ where y_i is the predicted test-day record for month i, b_j ($j = i-1$ or $i+1$) is the total regression coefficient, and x_j is the test-day record of the preceding $(i-1)$ or succeeding $(i+1)$ month. B_{i-1} and B_{i+1} are the intra-herd regression coefficients associated with the intra-herd prediction equation $y_i = \mu_i + B_j(x_j - \mu_j)$, where μ_i and μ_j are the herd averages for the i^{th} and j^{th} test-day records.

TABLE 3

Total and intra-herd regression factors for estimating a missing monthly test-day record when both the preceeding and succeeding test-day records are known [a]

Test-day record	Total regression			Intra-herd regression	
	a_i	b_{i-1}	b_{i+1}	B_{i-1}	B_{i+1}
2 from 1 and 3	2.8	.31	.69	.29	.67
3 from 2 and 4	1.0	.45	.56	.44	.56
4 from 3 and 5	1.9	.49	.47	.48	.46
5 from 4 and 6	2.8	.40	.56	.39	.54
6 from 5 and 7	1.6	.47	.51	.45	.51
7 from 6 and 8	1.5	.54	.40	.53	.39
8 from 7 and 9	0.8	.53	.47	.51	.47
9 from 8 and 10	−1.5	.54	.43	.54	.42

[a] a_i is the intercept of the prediction equation $y_i = a_i + b_{i-1}x_{i-1} + b_{i+1}x_{i+1}$, where b_{i-1} and b_{i+1} are the total regression coefficients associated with x_{i-1} and x_{i+1}, the test-day records of the preceding and succeeding months, respectively. B_{i-1} and B_{i+1} are the intra-herd regression coefficients associated with the within-herd prediction equation $y_i = \mu_i + B_{i-1}(x_{i-1} - \mu_{i-1}) + B_{i+1}(x_{i+1} - \mu_{i+1})$, where μ_i, μ_{i-1}, and μ_{i+1} are the herd averages for the test-day records of months of lactation i, $i-1$, and $i+1$, respectively.

known. Table 3 presents the regression factors for the case when both the preceding and succeeding monthly records are known. It is apparent that except for the latter situation the total and intra-herd regressions, in general, are not in close agreement. Then the bias of the total regression procedure relative to the intra-herd procedure depends on the known record as well as the herd and population monthly averages. Since the difference between B_j and b_j is numerically small, most of the bias depends on the differences between the herd and population averages for the monthly records.

Suppose, as an example, that the population average test-day record for the fifth month of lactation is 46.7 and for the fourth month, 51.7. Further suppose that the herd averages for the herd in which a missing record is to be estimated are 35.0 and 30.0 for the fourth and fifth months, respectively. Now suppose that the fifth monthly record of a cow in this herd is 31.0 and that the fourth record is missing. The three estimates are

$$y_{41} = 35.0 + .85(31.0 - 30.0) = 35.85$$
$$y_{42} = 8.7 + .92(31.0) = 37.22, \text{ and}$$
$$y_{43} = 1.11(31.0) = 34.41.$$

Assuming y_{41} is the best unbiased estimate, the biases are $d_{12} = 1.37$ and $d_{13} = -1.43$. These biases are about 4% of the best estimate.

The total and intra-herd correlations between the estimated missing monthly records and the actual records are given in Table 4. The total and intra-herd correlations are not appropriate, however, for comparing the accuracy of the two prediction procedures, VanVleck and Henderson (4). The relative efficiency of the two procedures can be measured by the ratio of their residual variances. The residual variance of the procedure, ignoring herd effects, is denoted by V_1 and that of the intra-herd procedure by V_2. Since it is expected that the intra-herd procedure has the smaller variance, the relative efficiency is measured by $E = (V_1/V_2)(100)$. The relative efficiencies, as well as the residual standard

TABLE 4

Total and intra-herd correlations between estimated and actual missing monthly test-day records, residual standard errors of the estimates, and the relative efficiency of total regression and intra-herd regression estimates [a]

Preceding record known						Succeeding record known						Preceding and succeeding known					
Monthly record estimated	R_1	$V_1^{1/2}$	R_2	$V_2^{1/2}$	E	Monthly record estimated	R_1	$V_1^{1/2}$	R_2	$V_2^{1/2}$	E	Monthly record estimated	R_1	$V_1^{1/2}$	R_2	$V_2^{1/2}$	E
2	.72	9.66	.60	9.13	112	1	.72	8.23	.60	7.87	109	2	.89	6.45	.83	6.38	102
3	.86	7.01	.80	6.81	106	2	.86	7.02	.80	6.82	106	3	.92	5.56	.88	5.50	102
4	.88	6.15	.82	5.92	108	3	.88	6.67	.82	6.50	105	4	.92	5.05	.88	4.99	102
5	.86	6.12	.79	5.92	107	4	.86	6.61	.79	6.38	107	5	.91	4.79	.87	4.73	103
6	.87	5.41	.82	5.32	103	5	.87	5.73	.82	5.53	107	6	.92	4.36	.88	4.33	101
7	.87	5.14	.83	5.03	104	6	.87	5.48	.83	5.24	109	7	.91	4.30	.88	4.26	102
8	.82	5.95	.78	5.81	105	7	.82	5.96	.78	5.62	112	8	.90	4.50	.88	4.46	102
9	.80	6.27	.77	6.12	105	8	.80	6.24	.77	5.83	115	9	.88	4.90	.87	4.84	102
10	.77	7.22	.75	7.11	103	9	.77	6.70	.75	6.40	110	..	....	.....	....	.....	.....

[a] R_1 is the correlation ignoring herd effects between the predicted and actual test-day record. $V_1^{1/2}$ is the residual standard deviation from estimation ignoring herd effects. R_2 is the intra-herd correlation between the predicted and actual test-day record. $V_2^{1/2}$ is the residual standard deviation from intra-herd estimation. E is the ratio $(V_1/V_2)(100)$ which expresses the relative efficiency of total regression to intra-herd regression prediction of missing test-day records.

deviations, are also presented in Table 4. The relative efficiencies indicate that the intra-herd procedure is only slightly better for nearly all months. When both adjacent records are known, the efficiencies of the two procedures are very similar. These comparisons indicate that the increased accuracy of the intra-herd procedure probably does not overcome the disadvantage of requiring the herd averages, which are difficult and expensive to obtain by records processing centers.

CONCLUSIONS

The intra-herd regression method is the best of the three procedures considered for predicting a missing monthly test-day record from a preceding or succeeding test-day record. The slight additional accuracy of prediction, however, is outweighed by the disadvantage that herd averages for the monthly test day records are required. Since population averages are easier to obtain and are easier to use than herd averages, prediction ignoring herd effects of missing monthly test-day records is preferable to intra-herd regression estimation.

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VARIANCE AND COVARIANCE COMPONENTS IN PART LACTATION MILK AND FAT RECORDS

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SUMMARY

The monthly test-day milk and fat records of 1,526 Holstein cows in 232 herds artificially sired by 145 different sires were analyzed to obtain estimates of sire, herd, sire by herd, and error components of variance for the ten test days and for the sum of ten test days and to obtain estimates of sire, herd, sire by herd, and error components of covariance between the monthly records and between the monthly records and the sum of ten test-day records. Although the variances of the estimates of interaction components of variance are not known, the results of the analysis indicate that the sire by herd components of variance are approximately zero. This would suggest that the sire by herd components of covariance are also negligible. It is felt, however, that more data should be analyzed, in order to substantiate or negate these results, since the number of records in the present analysis is small.

The lack of sire by herd interaction in dairy production data has been reported by Hickman and Henderson (6), Legates *et al.* (7), and Wadell and McGilliard (12) for complete lactation records. Sire by herd interaction components of variance and covariance for monthly records have not received much attention. Searle (10) found no appreciable sire by herd components from an analysis of New Zealand Jersey records. The presence or absence of an interaction component is quite important if records are analyzed according to a one-way classification model, after first taking the records as deviations from herd averages, or if selection index estimates of breeding value are to be obtained by procedures presented by Henderson (4), Lush (8), and Hazel (2).

Expressing records as deviations from herd averages removes the herd effect, except for sampling error, but does not adequately remove the herd by sire interaction effect. If the interaction component is zero, then deviations may be analyzed safely. Otherwise, a substantial error may be introduced. Also, the denominator of the part record regression of breeding value on part record daughter average used for estimating breeding value of a sire should contain the interaction component if the sire by herd interaction is present. The purpose of this study was to estimate the interaction components of variance and covariance for monthly test-day records of Holstein cows.

DATA

The DHIA records for this study were from 1,526 Holstein cows sired by 145 sires used in artificial insemination (A.I.) in 232 herds in five New York counties. Ten monthly test-day records were available for each cow's lactation, beginning with the 1st mo. of lactation and ending with the 10th mo. The sum

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of the ten test-day records which made up a cow's record was defined to be a complete lactation record. The sample of monthly test records was obtained from November, 1957, through December, 1959. The monthly records were corrected for age at calving and season of calving, with ratio factors described by VanVleck and Henderson (11).

ANALYSIS

The data were analyzed according to Henderson's (3) Method 1. The linear model, Model II of Eisenhart (1), assumed for each monthly record and for the complete record was

$Y_{ijk} = \mu + h_i + s_j + c_{ij} + e_{ijk}$ where

Y_{ijk} is the age and season corrected record of the k^{th} daughter of the j^{th} sire in the i^{th} herd,

μ is a fixed population parameter,

h_i is an effect common to the i^{th} herd,

s_j is an effect common to the j^{th} sire,

c_{ij} is an effect common to the ij^{th} sire-herd subclass, and

e_{ijk} is a random error effect associated with the ijk^{th} record.

Further it is assumed that the h_i, s_j, c_{ij}, and e_{ijk} are independently distributed with zero means and variances σ_h^2, σ_s^2, σ_c^2, and σ_e^2, respectively.

Sums of squares and cross-products of the monthly records were computed and equated to their expectations, to obtain estimates of components of variance and covariance. The actual computing procedure, Henderson and Searle (5), was as follows: (The symbols are defined in Table 1.)

$$\hat{\sigma}_{e_r e_{r'}} = (T - C)/(N - c),$$

$$R_a = H - CF - (h - 1)\,\sigma_{e_r e_{r'}}$$

$$R_b = S - CF - (s - 1)\,\hat{\sigma}_{e_r e_{r'}}$$

$$R_{ab} = C - H - S + CF - (c - h - s + 1)\,\hat{\sigma}_{e_r e_{r'}}$$

$$\hat{\sigma}_{e_r e_{r'}} = \left[\frac{R_a\left(\frac{k_1 - k_{21}}{N - k_{21}}\right) + R_b\left(\frac{k_2 - k_{12}}{N - k_{12}}\right) - R_{ab}\left(1 - \frac{k_1 - k_{21}}{N - k_{21}} - \frac{k_2 - k_{12}}{N - k_{12}}\right)}{-(N - k_1 - k_2 + k_3)}\right]$$

$$\hat{\sigma}_{h_r h_{r'}} = (R_a + R_{ab})/(N - k_{21}) - \hat{\sigma}_{e_r e_{r'}} \text{ and}$$

$$\hat{\sigma}_{s_r s_{r'}} = (R_b + R_{ab})/(N - k_{12}) - \hat{\sigma}_{e_r e_{r'}}$$

TABLE 1

Definitions of symbols used in computing procedure [a]

N = total number of observations = 1,526	
c = number of filled subclasses = 1,204	
h = number of herds = 232	
s = number of sires = 145	
$k_1 = \sum_i n_{i.}^2/N = 12.0406$	$k_{12} = \sum_i \frac{\sum_j n_{ij}^2}{n_{i.}} = 326.6691$
$k_2 = \sum_j n_{.j}^2/N = 40.7903$	
$k_3 = \sum_i \sum_j n_{ij}^2/N = 1.6880$	$k_{21} = \sum_j \frac{\sum_i n_{ij}^2}{n_{.j}} = 182.2050$
T = total sum of squares or cross-products	$= \sum_i \sum_j \sum_k Y_{ijkr} Y_{ijkr'}$
C = subclass sum of squares or cross-products	$= \sum_i \sum_j \frac{Y_{ij.r} Y_{ij.r'}}{n_{ij}}$
H = herd sum of squares or cross-products	$= \sum_i \frac{Y_{i..r} Y_{i..r'}}{n_{i.}}$
S = sire sum of squares or cross-products	$= \sum_j \frac{Y_{.j.r} Y_{.j.r'}}{n_{.j}}$
CF = correction factor for analysis of variance or covariance	$= \frac{Y_{...r} Y_{...r'}}{N}$

[a] r refers to the monthly record or to the complete record, i.e., $r = 1, \ldots, 10$ for the monthly records and $r = 11$ for the complete record. When $r = r'$ the quadratic is a sum of squares and when $r \neq r'$ the quadratic is a sum of cross-products.

The estimates are estimated covariance components if $r \neq r'$ and estimated variance components if $r = r'$. Estimates of total variances or covariances were obtained by summing the sire, herd, sire by herd, and error components. Negative values were included in the sum and were not set to zero.

RESULTS AND DISCUSSION

Estimates of (1) the variances of monthly test-day records, (2) the variance of the sum of ten monthly test-day records, (3) the covariances between individual monthly test-day records, and (4) the covariances between individual monthly test-day records and the sum of ten monthly test-day records are given in Table 2 for milk records and in Table 4 for fat records. The total variances of milk and fat records appear to follow a similar pattern over time, with the exception of the first monthly test-day record. The monthly tests are most variable in the first 3 mo., reaching a peak at the second or third test day. Then the variance decreases until the 8th mo., when it is about half as large as

TABLE 2

Estimates of total variance and covariance for ten monthly test-day milk records and the sum of ten monthly test-day records [a]

	Month of lactation										
Month	1	2	3	4	5	6	7	8	9	10	Sum
1	125.7	106.2	98.9	86.6	76.5	65.3	50.3	38.1	24.4	13.3	686.1
2	.....	179.0	157.4	133.5	111.5	95.6	77.3	58.7	39.9	27.0	986.0
3	.....	.....	185.4	148.6	122.3	104.0	87.6	69.2	46.1	32.7	1,052.2
4	.....	.....	.....	157.0	122.3	105.3	89.7	72.3	50.8	36.1	1,002.3
5	.....	.....	.....	.....	130.4	106.9	91.7	75.3	55.0	40.8	932.8
6	.....	.....	.....	.....	.....	113.4	92.0	75.4	56.1	43.2	857.2
7	.....	.....	.....	.....	.....	.....	100.4	79.6	59.5	47.6	775.7
8	.....	.....	.....	.....	.....	.....	.....	95.9	78.6	65.8	709.5
9	.....	.....	.....	.....	.....	.....	.....	.....	101.8	89.5	601.8
10	.....	.....	.....	.....	.....	.....	.....	.....	.....	134.0	529.9
Sum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8,133.4

[a] Estimates for monthly records, or lactation yield may be obtained by multiplying the tabled values by $(30.5)^2$.

TABLE 3

Estimates of the percentage contributed by herd, sire, sire by herd, and error components of variance or covariance to the total variance or covariance of monthly test-day milk records [a]

	Month of lactation										
Month	1	2	3	4	5	6	7	8	9	10	Sum
1	28, 2	40,–1	42, 0	45,–3	47, 0	48,–6	53,–6	52,–4	65,–15	80,–65	44, 3
	3,67	3,58	5,54	4,53	4,49	4,53	5,48	6,46	9,41	10,76	4,55
2	.....	32,–4	36,–7	39,–11	43,–10	43,–12	46,–9	43,–10	46,–15	39,–29	39,–9
		3,69	4,67	4,69	4,63	3,66	4,59	4,63	6,63	7,84	4,66
3	.....	.....	31,–6	36,–9	40,–7	40,–9	41,–6	38,–5	39,–8	33,–27	37,–7
			5,70	5,68	5,62	5,64	6,60	6,62	8,61	9,86	5,65
4	.....	.....	.....	32,–5	37,–5	38,–8	38,–6	36,–2	36, 0	30,–11	37,–6
				4,69	4,63	4,65	4,65	5,61	6,58	6,75	5,65
5	.....	.....	.....	.....	33,–4	35,–6	35,–5	32,–4	33,–2	28,–6	37,–5
					4,67	4,68	4,66	4,67	6,63	7,71	5,64
6	.....	.....	.....	.....	.....	29,–4	32,–3	30,–1	30, 6	27,11	35,–5
						4,71	4,67	4,67	6,58	8,55	4,65
7	.....	.....	.....	.....	.....	.....	28, 1	28, 1	29, 5	27,–1	35,–3
							3,68	4,67	4,62	5,69	4,63
8	.....	.....	.....	.....	.....	.....	.....	19, 8	19,11	18, 3	30, 0
								3,69	3,67	5,75	4,65
9	.....	.....	.....	.....	.....	.....	.....	.....	13, 5	13,–2	27, 1
									3,79	4,86	5,68
10	.....	.....	.....	.....	.....	.....	.....	.....	.....	9,–9	21,–8
										3,98	5,81
Sum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	35,–5
											5,65

[a] The percentages of the total variance or covariance for the four components of variance or covariance are arranged

$$\hat{\sigma}_{h_r h_{r'}}, \quad \hat{\sigma}_{c_r c_{r'}}$$

$$\hat{\sigma}_{s_r s_{r'}}, \quad \hat{\sigma}_{e_r e_{r'}}$$

If $r = r'$ the estimates are of variance components; otherwise, the estimates are of covariance components.

during the peak month. Following the period of minimum variability the variance increases through the 9th and 10th mo.

As one would expect, the covariance between monthly test-day records is highest for adjacent months and decreases as the interval between the test days increases. The covariances between monthly test-day records and the sum of ten test-day records are largest for the 2nd or 3rd mo. and decrease until the 10th mo., when the covariances are about half the largest values.

The percentages of the total variances or covariances which the sire, herd, sire by herd, and error components of variance or covariance constitute are given in Table 3 for milk records and in Table 5 for fat records. It should be noticed that negative percentages occur in the tables. This occurs because of negative estimates of the components.

The actual components can be re-formed by obtaining the product of the percentage corresponding to the desired component and the total variance or total covariance. Some of the percentages given in the tables do not sum to 100% because of rounding.

It appears that the sire by herd components of variance are probably zero or of little importance for all the monthly test-day records, as well as for complete records. Six of the ten interaction components of variance for monthly fat records were negative, as were six of ten for monthly milk records. The positive values were relatively small, but generally were as large or larger than the sire components. The interaction component estimates for total production were negative. These results are in general agreement with those found by Searle (10), who found small, mostly positive, interaction estimates for 1 yr.'s data and small, mostly negative, interaction components for the previous year's records.

A noticeable time trend appears when the relative sizes of the four variance components are examined. The error and herd components are relatively constant for the first 7 mo. The herd component then becomes relatively smaller as the error component makes up a large part of the total. This indicates that the variability of the 9th and 10th monthly test-day records is largely unaccounted for by herd and sire effects.

The problem of what to do with negative estimates of variance components is unsolved. Sometimes it is suggested that they be set equal to zero. Scheffé (9), however, has suggested that this is not a particularly good solution to the problem, since the estimates are not unbiased and because the distributions of the estimates are difficult to obtain. Another approach would be to delete the interaction effect in the model and reestimate the other components. This would be, however, a result-guided procedure and hence would not seem applicable. It would seem that negative estimates should be reported, if for no other reason than to provide unbiased estimates when summaries of various studies are made.

The estimated sire by herd components of covariance as seen in Tables 2 and 4 are predominantly negative and small. This agrees with Searle (10), who found estimates of nearly half the interaction components to be negative and all to be relatively small. The relative smallness of these components would

TABLE 4

Estimates of total variance and covariance for ten monthly test-day fat records and the sum of ten monthly test-day records

	Month of lactation										
Month	1	2	3	4	5	6	7	8	9	10	Sum
1	2.95	1.94	1.65	1.40	1.18	1.00	.82	.71	.49	.34	12.48
2	.....	2.97	2.17	1.78	1.46	1.27	1.03	.84	.57	.38	14.41
3	.....	.....	2.68	1.86	1.48	1.29	1.10	.90	.60	.40	14.13
4	.....	.....	.....	2.12	1.51	1.32	1.12	.94	.68	.49	13.23
5	.....	.....	.....	.....	1.64	1.30	1.11	.95	.69	.53	11.85
6	.....	.....	.....	.....	.....	1.51	1.16	.99	.72	.56	11.12
7	.....	.....	.....	.....	.....	.....	1.36	1.03	.75	.60	10.08
8	.....	.....	.....	.....	.....	.....	.....	1.32	.98	.81	9.48
9	.....	.....	.....	.....	.....	.....	.....	.....	1.36	1.15	7.99
10	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.84	7.09
Sum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	11.87

TABLE 5

Estimates of the percentage contributed by herd, sire, sire by herd, and error components of variance or covariance to the total variance or covariance of monthly test-day fat records [a]

	Month of lactation										
Month	1	2	3	4	5	6	7	8	9	10	Sum
1	24,–2	37,–2	39,–8	44,–9	45,–5	46,–7	47,–1	37, 3	44, 1	37,–33	37,–5
	4,75	3,61	5,63	6,60	7,53	5,57	5,49	5,55	11,44	16,80	5,62
2	.....	29,–4	37,–8	42,–13	44,–11	45,–8	46,–4	42,–5	45,–1	31,–18	38,–7
		3,73	4,67	2,69	3,63	1,63	1,56	0,64	4,52	10,77	3,66
3	.....	.....	26,–2	37,–9	41,–7	41,–7	40,–1	37,–6	40,–4	25,–18	36,–6
			4,72	4,67	6,60	4,62	4,57	4,66	6,58	10,83	5,65
4	.....	.....	.....	32,–5	39,–5	40,–3	39,–3	36,–1	36, 3	24,–5	38,–6
				2,71	4,62	2,62	3,60	2,61	4,57	6,76	3,65
5	.....	.....	.....	.....	33,–5	36, 0	36,–2	34,–4	34,–2	24,–8	38,–5
					3,69	3,61	4,63	3,67	5,62	7,77	4,63
6	.....	.....	.....	.....	.....	28, 7	32, 4	30, 3	33, 5	25,13	36, 0
						1,64	2,62	2,65	3,59	6,56	3,62
7	.....	.....	.....	.....	.....	.....	25, 8	28, 3	30, 0	23,–9	35, 0
							2,66	3,66	3,67	5,81	3,62
8	.....	.....	.....	.....	.....	.....	.....	19, 5	21, 7	18, 0	29, 1
								2,74	3,69	4,78	3,67
9	.....	.....	.....	.....	.....	.....	.....	.....	13, 5	13,–5	27, 1
									2,80	3,90	4,68
10	.....	.....	.....	.....	.....	.....	.....	.....	.....	9,–14	19,–8
										2,104	5,85
Sum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	34,–4
											4,66

[a] The percentages of the total variance or covariance for the four components of variance or covariance are arranged

$$\hat{\sigma}_{h_r h_{r'}},\ \hat{\sigma}_{c_r c_{r'}},$$

$$\hat{\sigma}_{s_r s_{r'}},\ \hat{\sigma}_{e\ e_{r'}}.$$

If $r = r'$ the estimates are of variance components; otherwise, the estimates are of covariance components.

indicate that this cause of error in estimation of sire and error components of covariance from analysis of deviations from herd averages would be negligible. The tendency for the estimates to be negative may be an indication that the parameter covariance values are, in fact, negative (a fact which would not be easy to explain, since the variances evidently approximate zero), or that the negative estimates are caused by sampling error (the average number of observations per subclass is $\frac{1,526}{1,204}$). The small number of observations per subclass may increase the sampling error in the estimation of the interaction components, even though many subclasses are filled. The most efficient proportion of subclass numbers relative to number of subclasses is apparently unknown in the estimation of variance and covariance components. If it can be assumed that the interaction components of covariance are zero, then the estimates of sire and error covariance components can be obtained from analysis of deviations from herd or stable-mate averages. This assumption would be true if the interaction effects were zero, which is indicated by the negative or small estimates of the interaction components of variance. Thus, it appears reasonable to believe that the interaction components of covariance are zero as well as the interaction components of variance.

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EFFECT OF GENETIC TREND ON SIRE EVALUATION

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SUMMARY

The contemporary comparison evaluation of 235 Holstein sires used in artificial insemination having daughters whose first lactations began during the period 1951-1959 were compared with the same evaluations adjusted for trend in genetic merit of their naturally sired contemporaries. Correlations were computed between the adjusted and unadjusted comparisons for all sires, regardless of number of daughters, for the 38 sires having the largest number of daughters, and within contemporary time groups. All estimated correlations were near unity for both milk and fat yield. These estimates indicate that with the present relatively slow change in genetic merit the trend in genetic merit need not be considered in the evaluation of contemporary young sires. If comparisons are desired between sires used several years apart, then it may be necessary to adjust for genetic trend.

A recent paper, VanVleck and Henderson (5), described a procedure for measuring the trend in genetic merit of a dairy population consisting of artificially (A.I.) and naturally (N.S.) sired females. Application of the method to the New York Holstein DHIA population showed an increase in genetic merit of both fractions of the population. The procedure answers the academic question, "How rapid has been the increase in genetic merit due to artificial insemination?" A more practical question is, "How does the change in merit affect sire evaluation?" The purpose of this paper is to investigate this latter question.

DATA AND PROCEDURE

The contemporary comparisons for sires included in the measurement of genetic trend by VanVleck and Henderson (5) were adjusted for the seasonal genetic merit of naturally sired contemporaries by the procedure also described by VanVleck and Henderson (5), in which a more complete description of the data may be found. A total of 235 A.I. Holstein sires had contemporary comparisons with effective weights, W, (W is the sum of the weights of all within herd-year-season comparisons for a particular sire) ranging from 0.50 to 914.89. These contemporary comparisons, both adjusted and unadjusted, were also regressed toward zero according to the regression of true breeding value on estimated breeding value, $\frac{W}{W+17}$, where 17 is the ratio of the within-sire variance component to the between-sire variance component as estimated by VanVleck *et al.* (4) from a portion of the same A.I. data.

Correlations were computed between the adjusted and unadjusted contemporary comparisons for both milk and fat yield for all sires, regardless of effective weights and for sires having effective weights greater than 100.

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The comparison of sires used in different periods of time is usually purely one of interest. Of more practical concern is the comparison of sires which are candidates at about the same time for entry into the permanent stud. To compare the adjusted and unadjusted comparisons for sires in service at approximately the same time, the sires were grouped, first, into six periods, according to when their first daughter records were reported to the New York Dairy Records Processing Laboratory, and second, into smaller time segments of 19 seasons. The inclusive months of freshening for the 19 seasons and six periods are shown in Table 1, together with the number of sires in each grouping. Note

TABLE 1

Distribution of sires according to the time when their daughter records were first available in the DHIA Central Processing Laboratory

Season	Inclusive months	No. of sires	Period	Inclusive months	No. of sires
1	September 1950—February 1951	35	1	September 1950—August 1951	48
2	March 1951—August 1951	13			
3	September 1951—February 1952	18			
4	March 1952—August 1952	3	2	September 1951—February 1953	27
5	September 1952—February 1953	6			
6	March 1953—August 1953	4			
7	September 1953—February 1954	6	3	March 1953—August 1954	20
8	March 1954—August 1954	10			
9	September 1954—February 1955	14			
10	March 1955—August 1955	14	4	September 1954—February 1956	55
11	September 1955—February 1956	27			
12	March 1956—August 1956	6			
13	September 1956—February 1957	10	5	March 1956—July 1957	21
14	March 1957—July 1957	5			
15	August 1957—November 1957	17			
16	December 1957—March 1958	10			
17	April 1958—July 1958	11	6	August 1957—March 1959	64
18	August 1958—November 1958	19			
19	December 1958—March 1959	7			

that several seasons are included in each period. Correlations between the adjusted and unadjusted comparisons were computed on a within-time group basis, in order to determine the effect of trend in evaluation of contemporary sires.

RESULTS AND DISCUSSION

The estimated correlations between contemporary comparisons, unadjusted and adjusted for trend in genetic merit, are shown in Table 2. All the values are near unity, which indicate that the adjustment is probably not required in order to evaluate sires accurately. The correlations within time groups are slightly higher than the correlations ignoring time groups. This further implies that classification of contemporary sires will be little affected by trend in genetic merit.

All the correlations between the regressed contemporary comparisons and the regressed adjusted contemporary comparisons are near one for all groupings of the data.

TABLE 2

Correlations between contemporary comparisons adjusted for genetic trend and comparisons unadjusted for genetic trend

Classification	Milk yield		Fat yield	
	Not regressed	Regressed	Not regressed	Regressed
All sires	.97	.99	.99	.98
Sires within periods	.98	.99	1.00	.99
Sires within seasons	.99	.99	1.00	.99
Sires with effective weight >100	1.00	1.00	.99	.99

TABLE 3

Contemporary comparisons adjusted and unadjusted for trend in genetic merit of 38 sires with effective weights larger than 100

Code No.	Sum of weights	Contemporary comparison				Ranking			
		Unadjusted		Adjusted		Milk		Fat	
		Milk	Fat	Milk	Fat	(1)[a]	(2)[b]	(1)[a]	(2)[b]
		(lb.)							
1	106.0	1,267.8	39.0	1,220.4	37.1	3	2	3	1
2	163.4	1,433.3	44.7	1,206.8	36.1	1	3	1	2
3	122.2	1,144.0	38.4	989.3	32.2	4	4	4	3
4	215.6	1,295.5	33.3	1,232.8	30.7	2	1	5	4
5	360.9	864.0	40.0	627.0	28.5	7	8	2	5
6	209.0	938.1	31.5	814.1	27.5	6	6	6	6
7	610.7	771.3	28.5	643.8	23.4	8	7	9	7
8	318.8	730.7	29.2	574.8	23.3	10	10	8	8
9	374.5	1,048.9	30.6	891.3	23.1	5	5	7	9
10	914.9	41.6	26.7	− 83.3	21.3	22	21	10	10
11	695.5	625.4	24.5	518.5	20.6	11	11	11	11
12	418.7	238.4	23.3	142.2	19.8	16	16	14	12
13	359.4	734.5	23.5	592.0	17.2	9	9	12	13
14	104.7	573.5	23.4	355.3	14.9	13	15	13	14
15	101.3	− 13.6	16.2	−116.3	12.1	25	22	17	15
16	147.2	598.9	21.5	377.5	11.3	12	12	15	16
17	333.4	515.5	17.4	355.4	11.1	14	14	16	17
18	347.2	78.3	7.3	15.3	4.8	19	17	20	18
19	382.7	148.4	10.4	− 15.5	3.2	18	19	18	19
20	515.8	198.2	7.4	12.2	− 0.6	17	18	19	20
21	107.2	− 46.2	− 0.5	−152.9	− 4.4	26	23	22	21
22	394.8	−301.3	0.4	−467.2	− 6.0	29	29	21	22
23	109.4	67.2	− 3.4	− 37.8	− 6.7	20	20	28	23
24	290.7	−424.2	− 1.8	−549.4	− 7.0	35	33	24	24
25	136.1	486.2	− 2.3	356.2	− 7.4	15	13	26	25
26	262.7	−453.9	− 5.4	−527.3	− 8.5	36	32	30	26
27	310.3	1.6	− 2.5	−168.9	− 9.7	23	24	27	27
28	886.4	−411.3	− 1.9	−589.2	− 9.9	33	34	25	28
29	372.9	46.8	− 0.5	−171.1	−10.2	21	25	23	29
30	227.8	− 12.7	− 3.9	−231.1	−14.3	24	26	29	30
31	169.8	−344.8	−11.4	−449.6	−15.2	30	28	31	31
32	138.5	−412.0	−12.8	−493.7	−15.7	34	31	33	32
33	196.4	−112.3	−11.6	−360.3	−23.8	27	27	32	33
34	483.2	−400.3	−16.5	−589.4	−24.9	32	35	34	34
35	101.6	−275.8	−16.7	−481.6	−27.1	28	30	35	35
36	233.3	−489.2	−22.0	−691.7	−30.1	37	37	36	36
37	231.9	−587.9	−23.8	−829.4	−35.9	38	38	37	37
38	144.4	−358.6	−32.8	−643.9	−47.0	31	36	38	38

[a] Rank according to unadjusted contemporary comparison.
[b] Rank according to adjusted contemporary comparison.

As can be seen from Table 3, where the 38 sires are listed together with their adjusted and unadjusted contemporary comparisons for milk and fat yield, the rankings of sires used at differen periods of time can be influenced by trend in genetic merit. For example, if evaluation is on fat yield the contemporary comparison of Sire 2 is 5.7 lb. higher than for Sire 1, but if the values are adjusted for trend then Sire 1 is estimated to be 1.0 lb. better than Sire 2, a change of 6.7 lb. in the estimated difference between the two sires. An example where the change is even larger also concerns Sire 1, now compared to Sire 5. Unadjusted estimates for Sire 5 give a 1.0 lb. fat yield advantage. If the estimates are adjusted for trend, Sire 1 has an 8.6-lb. advantage, a change of 9.6 lb. in the estimated difference between them. This is an example of comparing a father, Sire 5, to a son, Sire 1, when the time interval is important. The changes in ranking for milk yield due to adjusting for trend do not appear as great as for fat yield.

It must be emphasized that these procedures for evaluating A.I. sires are not the ones used in New York State. These methods are merely used to estimate trend in merit and to investigate the effect of genetic trend on sire evaluation. The procedures used in New York are described in various papers, Henderson *et al.* (3), Henderson (2), and Heidhues *et al.* (1). It is felt, however, that the conclusions derived from this study are also applicable to the system actually used for sire evaluation in New York.

CONCLUSIONS

Results of this study indicate that trend in genetic merit has little importance in evaluation of contemporary sires. Evaluation disregarding trend is highly correlated with evaluation adjusted for trend.

If sires used in widely different time periods are to be compared, then trend in genetic merit probably should be considered.

The increase in genetic merit of the natural service population from which the data were drawn has been little more than 0.5% per year for the period 1951-1959. If the increase were more rapid, then the first conclusion above may not be correct and the second conclusion may be even more appropriate.

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PHYSIOLOGICAL STUDIES OF THE RUMEN WITH EMPHASIS ON THE ANIMAL FACTORS ASSOCIATED WITH BLOAT[1]

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SUMMARY

Several physiological processes were compared between animals with known bloat susceptibility fed a ration of fresh alfalfa tops.

Only stable IVI (ingesta volume increase) and salivary secretion and composition differed significantly between susceptible and nonsusceptible cows.

A second, more extensive study showed that the susceptible cows secreted significantly less saliva during rest and during feeding, with a significantly higher concentration of bicarbonate than the nonsusceptible animals. Whole ingesta from susceptible cows normally was higher in dry matter content and less dense (due to foaminess) than ingesta from nonsusceptible cows. No differences were found in salivary potassium, phosphate, sodium, and mucin; frequency and amplitude of rumen contractions; rate of gas production; total-, soluble-, and ammonia-nitrogen of rumen liquor; buffering capacity of ingesta and saliva; rumen pH and water consumption.

When fed chopped alfalfa hay, a nonsusceptible cow appeared to secrete significantly more saliva during rest, with a significantly lower concentration of potassium than the susceptible cows. Salivary bicarbonate, phosphate, sodium, mucin, and water consumption did not differ significantly. Whole ingesta dry matter content was normally greatest in the susceptible cows.

The physical nature of the ingesta is the most important characteristic distinguishing bloating from nonbloating animals. Saliva added to the rumen during periods of rest may exert an influence on this characteristic, we suggest, by preventing stable foam formation through reduced ingesta surface tension, and by reducing the concentration of agents responsible for foam stabilization through more complete fermentation.

Certain ruminants are more susceptible to bloat than others. According to Kephart (26), cattle are prone to the condition, whereas sheep, on the other hand, "nearly never bloat." Differences in susceptibility between the individuals of cattle herds and sheep flocks have been noted by many investigators (11, 24, 27, 29-31, 33, 38, 44).

Knapp and coworkers tested the bloat susceptibility of steer progeny from 13 bulls over a 2-yr. period. There were, they concluded, inherent differences between the progeny of different bulls in their ability to handle large quantities of feed without digestive disturbances, because progeny from certain bulls were observed to bloat more frequently than progeny from others.

Hancock (21) observed wide differences in bloat susceptibility between sets of twins and great similarities in this respect within twin-sets. He noted 60% of the bad bloaters to be the daughters of one bull, thus supporting the findings of Knapp and associates. Johns (24, 25) also noted differences between twin-

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pairs, both on pasture trials and in stall-feeding experiments. In discussing his 1954 trial, he states, "It appears from the results presented and from the literature that there are at least two major groups of factors in the etiology of bloat: a plant factor or factors usually associated with legumes, *Cruciferae*, and occasionally young ryegrass, and animal factors."

Elucidation of the mechanisms involved in bloat thus appears to necessitate an understanding of the means by which plant and animal factors combine to produce the condition. However, identity of these so-called factors is largely unknown.

Some physiological processes suggested as constituting animal factors are: salivary flow, salivary composition, fluid transfer from the rumen, rumen motility (particularly with respect to eructation efficiency), gas production, microbial population, rate of eating, rumen pH, and breakdown products of nitrogen metabolism (especially soluble protein). A survey of the literature reveals nearly a complete lack of attempts to compare any of these factors between bloat-susceptible and nonsusceptible animals. Two notable exceptions are the comparisons made by Johns (24), on rates of eating and fermentation rate comparisons by Hungate and coworkers (23). Most of the above factors were compared in the current studies.

EXPERIMENTAL PROCEDURE

Relationship of certain physiological processes to bloat susceptibility. Accurate knowledge of individual bloat susceptibility is a prerequisite to comparative studies of physiological processes. Susceptibility was classified as previously described (33). Results of this study are summarized in Table 1.

Animals were selected for the current studies on the basis of per cent times bloated, maximum intrarumen pressure, and bloat index. Animals with large positive indices were classified as bloaters, whereas a large negative index indicates nonsusceptibility.

Cow 80 was included as a reversing type (33) because of the possibility that some process could be observed to change from periods of susceptibility to nonsusceptibility. Howver, no reversal occurred and this animal bloated well throughout the experimental period.

Because susceptibility was found to be quite predictable, reference is made to each animal on the basis of this classification, regardless of whether she bloated in the current studies or not. Each of the animals was fitted with a large-diameter fistula, as previously described (32).

TRIAL I

Trial I consisted of seven experimental periods, each of three days' duration, during which a bloat-susceptible animal was compared with a nonsusceptible subject. The pairs were rotated at the end of each three-day period. Thus, each nonbloater was compared with each of two bloaters three or four times.

Alfalfa tops, 4 to 6 in. in length, were fed twice daily. The amount offered during the 30-min. feeding interval of the motility studies was varied from one experimental period to the next; however, each bloater-nonbloater pair received

TABLE 1

Comparison of individual bloat susceptibility

Cow	Average [a] feed intake [Fa]	No. of [b] tests	% Times bloated < 20 mm. Hg	Max. intraruminal press.[c] Observed (Av.) [Ta]	Predicted [d] (Av.) [Tp]	Max. attained	Bloat index [Ta-Tp]
	(lb.)				*(mm. Hg)*		
				1958			
37	31	25	20	15	9	40	+ 6
84	34	27	56	19	10	25	+ 9
80	38	26	62	20	11	45	+ 9
104	32	26	15	11	11	22	+ 1
19	52	27	4	4	17	21	−13
8	41	24	58	22	12	38	+10
968	49	25	0	0	18	6	−18
139	37	26	0	4	12	12	− 8
961	37	24	40	17	10	35	+ 7
				1959			
37*	30	24	71	24	11	41	+13
84*	31	23	91	28	11	47	+17
80*	46	23	13	5	19	39	−14
19	47	16	25	10	17	45	− 7
968*	47	23	4	2	21	36	−19
139	33	23	39	18	13	38	+ 5
961	39	23	40	19	15	40	+ 4
169	44	23	22	7	16	33	− 9
966*	45	23	0	2	19	11	−17
29	40	23	52	18	16	38	+ 2
94	28	23	56	20	11	33	+ 9
75*	36	23	87	26	13	38	+13

* Fistulated.

[a] Average feed intake for eight control cows in 1958 was 39-41 lb. and for 11 control cows in 1959 was 38-39 lb. [Fc].

[b] Any feeding period during which at least one cow showed an intraruminal pressure of 20 mm. Hg or more was considered to be one test.

[c] The average observed maximum intraruminal pressure for eight control cows in 1958 was 12-13 mm. Hg and 14-17 mm. Hg for 11 control cows in 1959 [T].

[d] The maximum intraruminal pressure predicted from the feed consumption calculated from: $\frac{Fa}{Fc} \times T = Tp$.

identical amounts and the total consumption per day remained constant within each experimental period. The alfalfa tops were harvested daily by a flail-type harvester and transported and stored in a two-wheel trailer. The trailer was kept covered during the day with a tarpaulin to retard drying and wilting of the alfalfa tops. Water consumption was measured daily.

During Day 1, rumen contractions were measured, both in a bloater and in a nonbloater, as described earlier (34). Each motility study was composed of four divisions: a 15-min. adjustment period, a 15-min. prefeeding period, a 30-min. feeding period, and a 2-hr. post-feeding period. The motility pattern was recorded continuously throughout the 3-hr. period and was continued for greater lengths of time only if an animal was still experiencing bloat at the end of the 2-hr. post-feeding period.

During Day 2 the bloater-nonbloater pair employed the previous day was

again used. Prefeeding rumen samples were collected, after stirring the rumen contents in situ with the arm. A 50-ml. aliquot of strained rumen liquor from each animal was analyzed for various nitrogen fractions, according to the method of Moore and King (35).

Buffering capacity of the liquor was measured by direct titration using 1 *N* HCl and 1 *N* KOH under an atmosphere of 100% CO_2. The pH was measured immediately after collection with a Model 125 Photovolt pH meter, using a glass electrode and a calomel reference electrode.

After collection and analysis of the prefeeding sample, the rumen of each animal was evacuated and resting and feeding salivary flows were measured, as previously described (34). A 50-ml. aliquot of the saliva was frozen for later analysis of sodium, potassium, inorganic phosphate, and alcohol-insoluble substances (mucin). A 25-ml. sample was also collected at the cardia in a test tube, quickly stoppered, and analyzed for CO_2 concentration in a Van Slyke apparatus.

Salivary sodium and potassium concentrations were determined on a Beckman Model B spectrophotometer equipped with a flame attachment. The sodium was read at 589 mμ and potassium at 768 mμ. Inorganic phosphate concentrations were determined by the method of Fisk and Subbarow (20); an Evelyn colorimeter equipped with a 660-mμ filter was used in these determinations.

Hawk, Oser, and Summerson (22) describe a technique for precipitating mucin in the presence of 95% ethanol. Although it is unlikely that pure mucin is isolated by this procedure, contaminating material should not be large, thereby permitting a comparison of mucilaginous material between animals. With these limitations in mind, aliquots of the saliva were so analyzed for mucin.

Rumen pH measurements were made during the afternoon. The ingesta was stirred in situ and a 500-ml. sample was placed immediately on a portable pH meter. The pH measurements began immediately before feeding and continued at 15-min. intervals for 1 hr. post-feeding. Following these measurements, the rumen contents were sampled and treated as above, with ingesta volume increase (IVI) measurements also being made. The IVI was determined according to the method of Lindahl *et al.* (29).

Dry matter determinations were made on all ingesta, salivary, and bolus samples.

On Day 3, total rumen gas production was measured in the bloater-nonbloater pair used during the two previous days. Methods of gas collection were those previously employed at this station (11).

Further studies of salivary flow and composition on fresh alfalfa tops (Trial II). The results of Trial I indicated that an important difference existed, with regard to salivary flow and composition, between susceptible and nonsusceptible animals. Thus, a second study was carried out in an attempt to investigate this relationship more thoroughly.

Five of the animals from the initial study were compared for 40 days; a sixth animal, Cow 37, was removed from the study on the 19th day because of an irritated fistula. Daily water consumption was measured during the last 21 days of the study. Feed intake remained constant at 70 lb. alfalfa tops per head per day.

Each morning, the rumens of a bloater-nonbloater pair were evacuated; the rumen contents, placed in calibrated, galvanized garbage cans, were weighed; and the volume measured. Density values were calculated from these data.

Aliquots 200-300 g. in size were taken from the bolus material and ingesta for dry matter determinations. These samples were weighed 10-15 min. after collection; in the case of saliva, approximately 35 g. of saliva were used. Resting and feeding salivary rates were measured, as before. A 50-ml. aliquot was taken from this material for freezing and later analysis. Samples for bicarbonate determinations were collected and treated as before.

Relationships of salivary flow and composition to bloat susceptibility on chopped alfalfa hay (Trial III). Four fistulated cows were fed 15 lb. of U.S. No. 1 chopped alfalfa hay each day for a period of 19 days. A ten-day adjustment period, during which chopped alfalfa hay was fed, preceded the experiment. The experimental procedures were those employed in Trial II.

RESULTS

TRIAL I

The amount of bloat experienced during Trial I was quite disappointing; out of 26 possibilities, seven cases of slight to moderate bloat were observed. The herbage being fed was capable of producing bloat, however, because two cows not on trial but fed free choice bloated regularly. It is not entirely clear why the incidence was so low in the experimental animals. In a previous study (33), intake was found to be of little importance to the development of bloat. Thus, it would appear that removal of the ingesta for short periods tended to depress bloat. Further, Colvin and associates (14) have shown that satisfactory experimental bloat can be produced on a daily intake of 60 lb. of bloatable herbage. Only a slight increase in the incidence of bloat occurred when daily intake was increased to 70 lb. in the present investigation.

Rumen motility. A tabular comparison of total rumen contractions is presented in Table 2. These data show no essential differences in frequency of

TABLE 2

Comparison of rumen motility between bloaters and nonbloaters

	No. of contractions/15 min.				Contractions/lb fed/15 min.			
Cow	N [a]	Total	Retic. [b]	Eruct. [c]	E/R [d]	Total	Retic.	Eruct.
				Nonbloaters				
968	9	251	150	100	1:1.5	9.1	5.5	3.6
966	10	268	167	99	1:1.7	9.2	5.7	3.4
				Bloaters				
37	9	256	156	102	1:1.5	10.7	6.5	4.3
80	9	260	162	102	1:1.6	9.7	6.0	3.9
84	11	326	201	127	1:1.6	11.2	6.9	4.3

[a] Number of observations.
[b] Reticular contractions.
[c] Eructation contractions.
[d] Ratio of eructation to reticular contractions.

rumen contractions between the susceptible and nonsusceptible cows. Cow 84 exhibited a greater total frequency because she experienced more bloat than any of the others. Although absolute reticular and eructation frequencies and frequencies per pound of feed are greater in this cow, the total ratio of eructation to mixing contractions remained unchanged. Types of ratios, i.e., 1:2, 1:1, etc., showed no differences except when moderate bloat was experienced. Under such conditions, a greater number of 1:1 ratios were observed.

TABLE 3

Comparison of amplitude of mixing contractions between bloaters and nonbloaters before and after feeding

Cow	No. of observations	Average amplitude/15 min. (cm. H_2O)		
		15 Min. before feeding	30 Min. post-feeding	75 Min. post-feeding
		Nonbloaters		
968	4	13.5	17.6	14.1
966	5	7.7	15.7	13.3
Average		10.6 ± 1.22	16.7 ± 1.31	13.7 ± 0.99
		Bloaters		
37	3	9.8	13.4	11.7
80	2	7.8	14.8	12.7
84	5	8.1	10.6	9.4
Average		8.4 ± 0.41	12.3 ± 0.90	10.8 ± 0.85

The nonbloaters appeared to posses a greater amplitude of mixing contractions than did the bloat-susceptible cows (Table 3). Cow 968 showed contractions nearly twice as strong as 966 during the 15-min. period before feeding, yet both are nonbloaters. In fact, Cow 966 showed a lower contraction strength during this period than did the bloat-susceptible animals. In spite of this, values during the two post-feeding measurement periods were consistently greater for these two cows. A statistically significant difference exists during the latter two periods ($P < 0.05$ and $P < 0.01$, respectively) between the bloater and nonbloaters when differences in intrarumen pressures were not considered.

A comparison of mean amplitudes with mean intrarumen pressures, both in the bloated and nonbloated conditions, shows quite clearly that the differences are actually a reflection of a greater intrarumen pressure during bloat and not a physiological difference, as one might be led to believe. Contraction amplitude diminished with increasing intrarumen pressure, regardless of bloat susceptibility; therefore, is not correlated with susceptibility (Table 4).

Nitrogen content of rumen liquor. Nitrogen values varied widely from day to day; however, average total concentrations were comparable to those reported by Moore and King (35). No significant differences were found with respect to those nitrogen fractions investigated (Table 5).

Buffering capacity of ingesta and saliva. Turner and Hodgetts (42) equilibrated ingesta with varying pCO_2 and nitrogen and showed a considerably

TABLE 4

Effect of bloat on amplitude of mixing contractions

Cow	Classification [a]	No. of observations	Average amplitude/15 min. (cm. H_2O)					
			15 Min. before feeding		30 Min. post-feeding		75 Min. post-feeding	
			C.A.[b]	IRP [c]	C.A.	IRP	C.A.	IRP
			No bloat					
966	NB	5	7.7	−6	15.7	−5	13.3	−5
968	NB	2	14.7	−8	22.1	−8	20.7	−4
37	B	2	9.8	−6	13.4	−4	11.7	−3
80	B	2	7.8	−4	14.8	−4	12.7	−2
84	B	3	8.2	−8	11.8	−2	11.3	−1
			Bloated					
968*	NB	2	11.9	−3	13.1	−4	10.8	7
37	B	1	10.0	−6	11.4	6	9.1	8
84	B	2	7.8	−5	8.8	6	6.5	13

* Note: Cow 968, classified as a nonbloater, actually had a slight positive intrarumen pressure on two occasions during Trial I.

[a] NB = Nonbloater, B = Bloater.

[b] Contraction amplitude.

[c] Intrarumen pressure in cm. H_2O.

flattened titration curve with increasing pCO_2. Therefore, the buffering curves obtained under 100% CO_2 do not reflect true buffering capacity. They should, however, permit a comparison of the buffering capacity of the ingesta and saliva from different animals.

The prefeeding and post-feeding ingestas appear to be equally well buffered against the addition of 1 *N* HCl, with no apparent differences in buffering capacity between animals. In most cases, the prefeeding ingesta appeared to possess slightly less ability to buffer against the addition of 1 *N* KOH than did the post-feeding ingesta. In all cases, saliva exhibited a greater buffering ca-

TABLE 5

Comparison of gas production, total nitrogen in prefeeding ingesta, and rumen pH values between bloat-susceptible and nonsusceptible cattle fed fresh alfalfa tops

Cow	Total gas production [a] (liter/3 hr.)	Total prefeeding nitrogen (mg/100 ml.)	Rumen pH at 15-min. intervals from initiation of feeding [c]				
			P.F.[b]	15	30	45	1 hr.
			Nonbloaters				
968	129.6	117.2	7.25	6.92	6.68	6.61	6.60
966	129.8	87.4	7.57	7.05	6.72	6.74	6.76
			Bloaters				
37	.....	93.5	7.41	7.11	7.00	6.94	6.91
80	127.8	144.1	7.42	7.09	7.00	6.80	6.89
84	141.7	97.3	7.34	6.85	6.76	6.63	6.64

[a] Thirty-five pounds of alfalfa tops were consumed during a 30-min. feeding period.

[b] Prefeeding pH values.

[c] Each an average of seven observations.

pacity than did ingesta from the same animal. This was true up to approximately pH 5.3; at which point salivary buffering ability decreased quite rapidly. In no instance was there a significant difference in salivary buffering capacity between animals.

Rumen pH. Rumen pH has been reported to be lower in bloating than in nonbloating cattle (6). The results of this trial are not in agreement with those findings (Table 5). In fact, no differences in pH were found, even though the bloaters were experiencing some bloat. These findings support the titration data and suggest that rumen pH is not of primary importance in the development of bloat.

Dry matter. Per cent dry matter of the saliva was similar for all animals (0.97); however, dry matter content of the bolus material differed by 1.34%, indicating that more saliva was present in the boluses from nonbloating animals. This was later substantiated in the salivary studies (Trial II).

Stable ingesta volume increase (SIVI). Stable ingesta volume increase (SIVI) showed a remarkable difference (15.75%). Mean values were 0.5 and 16.2% for the nonbloaters and bloaters, respectively. These differences show quite clearly that even in the absence of marked bloat there were still factors present which could maintain a stable foam.

Water consumption. Average daily water consumption was similar for all animals, with wide daily variations (Table 9).

TABLE 6

Dry matter consumed, dry matter of ingesta, volume and weight of rumen contents, and ingesta density

Cow	No. trials	Dry matter		Rumen content		
		Consumed	Whole ingesta	Volume	Weight	Density
		(lb.)	*(%)*	*(liters)*	*(kg.)*	
			Nonbloaters			
Alfalfa tops						
968	19	12.22	7.30	47	44.8	0.9536
966	17	12.14	6.79	70	67.5	0.9656
			Bloaters			
80	12	12.40	8.35	42	39.2	0.9428
37[a]	4	10.97	7.23	48	47.1	0.9840
84	9	11.60	7.88	67	58.9	0.8772
75	11	12.44	7.28	44	43.3	0.9774
Chopped alfalfa hay						
			Nonbloaters			
968	12	12.41	7.82	56	54.2	0.9722
			Bloaters			
37	12	10.10	7.96	55	54.3	0.9873
80	12	12.34	10.16	62	60.1	0.9622
75	12	12.51	8.46	52	51.2	0.9886

[a] Cow 37 consumed 60 lb. of alfalfa tops daily; others consumed 70 lb. daily.

Gas production. In general, gas production was nearly identical for all cows (Table 5). One to 2 hr. post-feeding it averaged approximately 61 liters per hour—a rate comparable to those reported by Cole *et al.* (11) and Colvin (13). For the entire 3-hr. period, production averaged 44 liters per hour as production rapidly declined after about 2 hr.

TABLE 7

Comparison of salivary rates and composition between bloat-susceptible and nonsusceptible cattle fed fresh alfalfa tops

Cow	Secretory rates			No. of observations	Composition (*mg/100 ml.*)				
	Resting (*ml/min*)	Feeding [a] (*g/sec*)			HCO_3 [b]	HPO_4	Na	K	Mucin
		1st	2nd						
				Nonbloaters					
968	72.9	2.73	2.09	19	109.2	46.4	426	21.2	252
966	61.3	3.25	2.51	17	102.5	73.0	425	27.0	329
Average	67.5	3.0	2.3		105.7	59.0	425.5	23.9	288
S.E. [c]	2.5	0.05	0.06		1.5	3.4	12.0	1.9	26.8
				Bloaters					
37	37.5	2.19	1.75	4	106.6	34.5	337	27.3	219
80	53.2	2.33	1.66	12	117.0	46.1	423	21.3	279
84	51.6	2.76	1.85	9	111.3	46.1	429	23.3	290
75	58.5	2.81	2.05	11	110.0	42.9	423	26.2	279
Average	52.7**	2.6**	1.8**		112.6**	43.5**	413.2	23.8	275
S.E. [c]	1.9	0.05	0.05		1.85	1.6	10.8	1.0	25.0

** Significant at 1% level.

[a] Four 500-g. portions of alfalfa tops were fed during the first feeding period. Twenty minutes later four 500-g. portions were again fed (second feeding).

[b] m*M* CO_2/liters.

[c] Standard error of the mean.

Rate of salivary flow. Comparison of salivary flow between types during this initial study revealed some important differences. The nonbloaters secreted 5% more saliva during rest and 28% more during feeding than did the bloaters.

The resting rates (nonbloaters, 65.9 ± 6.7 ml/min; and bloaters, 62.6 ± 5.2 ml/min) are in all probability too high, since Ash and Kay (1) have shown that insertion of a cannula into the cardia of sheep produces an immediate and copious response in parotid secretion. This does not invalidate these data for the purpose of making comparisons between animals, however, since all animals were treated the same and great care was taken at all times to keep the animals on a rigid routine in an attempt to minimize unknown variables.

The feeding rates were similar to those reported by Balch (3). He reports a secretion rate of 4.5 to 5.5 lb. saliva/10 lb. fodder beet, which amounts to 450-550 g. saliva/kg feed. Cows 966 and 968 secreted approximately 470 g. saliva/kg green alfalfa tops, whereas Cows 80 and 84 secreted only 340 g/kg. Balch calculated that his animals salivated at the rate of 3.73 lb/10 min. (169 g/min.). This is somewhat greater than the 142 g/min and 107 g/min secreted by the nonbloaters and bloaters, respectively, in these trials. Balch did not,

TABLE 8

Comparison of salivary rates and composition between bloat-susceptible and nonsusceptible cattle fed chopped alfalfa hay

Cow	Secretory rates		No. of observations	Composition (mg/100 ml.)				
	Resting (ml/min)	Feeding (g/sec)		HCO_3	HPO_4	Na	K	Mucin
				Nonbloaters				
968	78.7	3.00	12	119.93	40.1	353	17.2	311
Average	78.7	3.0		119.93	40.1	353.3	17.2	311
S.E.[a]	4.1	0.20		1.58	1.7	6.33	0.9	1.8
				Bloaters				
37	75.2	2.65	12	125.20	30.6	346	32.5	355
80	43.8	2.32	12	111.27	44.0	344	48.2	317
75	65.9	2.70	12	117.27	41.8	339	54.6	338
Average	61.6**	2.53		117.91	38.8	342.9	45.1*	337
S.E.[a]	3.8	0.04		3.2	1.5	6.41	7.5	25.9

* Significant at 5% level.
** Significant at 1% level.
[a] Standard error of the mean.

however, report the dry matter content of the fodder beet. The average dry matter content of the forage in this study amounted to 16%.

Composition of saliva. Salivary composition appeared to differ significantly between bloaters and nonbloaters with respect to bicarbonate ($P > 0.05$), potassium ($P > 0.05$), inorganic phosphorus ($P > 0.01$), and mucin ($P > 0.05$). Bicarbonate and potassium levels were greatest in the saliva of bloaters, whereas inorganic phosphate and mucin levels were greatest in that of the nonbloaters.

Further studies of salivary flow and composition on fresh alfalfa tops (Trial II). The bloat experienced in Trial II was confined to Cows 80 and 84. Throughout most of the study these animals exhibited very foamy, frothy ingesta, whereas ingesta from Cows 968, 966, and 75 were dark-green fluids displaying little or no foaminess. This is reflected in the ingesta density values (Table 6), both cows which bloated having lower density values than the nonbloating animals.

TABLE 9

Average daily water consumption (liters/24 hr.)

Trial	Cow					
	968	966	37	80	84	75
	Nonbloaters			Bloaters		
I (alfalfa tops)						
Mean	20	27	28	20	23	
Range	9–31	4–44	11–35	9–31	1–36	
II (alfalfa tops)						
Mean	15	18		18	20	26
Range	5–22	5–40		11–28	0–20	13–42
III (chopped alfalfa hay)						
Mean	28		34	30		40
Range	6–52		22–52	14–56		19–54

Shortly after feeding, Cow 968 was frequently observed to have a foamy ingesta. The foam was characteristically unstable, although the density values indicated some foam may still have existed in the prefeeding ingesta (Table 6).

Cow 75 was classified as a bloater; however, no bloat was observed in this animal throughout the experiment. Her ingesta was characteristically thin and watery in nature, in addition to the fact that her salivary rates were very similar to those of the nonbloaters. Thus, the values obtained from this cow probably should have been included with the nonbloater data. They were not, however, because of the earlier classification. This should be kept in mind when evaluating the results.

The per cent dry matter of the ingesta from the two bloating cows was somewhat greater than that from the nonbloaters, even though the amount of dry matter consumed was identical on any given day (Table 6). Volume and weight of the ingesta were not related to bloat, except as seen in the density values.

Resting salivary rates for Trial II are presented in Table 7. The bloaters were found to salivate at a significantly ($P < 0.01$) lower rate than the nonbloaters. Reduced rates of salivation by the bloaters was also observed during feeding, both at the initiation of feeding and 20 min. later (Table 7). The bloaters, on the average, secreted 14 and 20% less than the nonbloaters at the beginning and at the conclusion of the trials, respectively.

Analysis of the data for the first measurement period shows statistically significant differences for days ($P < 0.05$) and type ($P < 0.01$). Therefore, it was not possible to determine precisely whether the difference in mean secretory rates is due entirely to cow type or to the daily variations within cows. Analysis of the second period showed a similar situation. On the basis of the small standard errors of the means, the significance shown for type, and the consistently higher rates shown by the nonbloaters, it is suggested that secretory differences during feeding do exist between bloat-susceptible and nonsusceptible animals, but that large numbers of samples are necessary to evaluate these differences correctly.

Average composition of saliva is presented in Table 7. Statistical analysis of the various components reveals only inorganic phosphate and bicarbonate to be significantly different for type ($P < 0.01$). Sodium and mucin show significant daily variations regardless of type, whereas potassium shows no significance, with wide daily fluctuations for all cows. Since daily variation of inorganic phosphate shows significance at the 5% level, it was not possible to analyze the data further, although unusually high phosphate values shown by Cow 966 seem to exclude the significance attributed to type and indicate a high variability among cows. The lack of significance found in these more extensive data seems to invalidate the significance found for all constituents, except bicarbonate, of Trial I.

Average daily water consumption was comparable for all cows, though Cow 75 showed a somewhat higher average intake, possibly because she lost ingesta on several occasions as a result of fistula plug losses (Table 9).

Conclusions: (a) Bloat-susceptible cattle secrete significantly less saliva during rest and during feeding than do nonsusceptible cattle when fed an alfalfa-tops ration. (b) Saliva bicarbonate concentration is greater from bloaters than from nonbloaters; otherwise, composition does not vary significantly between types. (c) Dry matter content and density of whole rumen ingesta is normally highest and lowest, respectively, in bloaters. (d) Water consumption is not different between types.

Salivary flow and composition on chopped alfalfa hay (Trial III). This experiment was conducted in an attempt to determine whether type differences observed on an alfalfa-tops ration persisted on dry, nonbloatable herbage.

Rumen dry matter content of Cow 968 was somewhat lower than in the three bloater-type cows (Table 6). Since feed intake was identical, this difference may have been due to differences in salivary flow or in movement of fluid out of the rumen. Density values for all cows were comparable, with the exception of Cow 80. Her lower density value is probably the result of a high dry matter content which, in turn, presumably resulted from a low rate of salivation (Table 6).

Cow 968 showed a significantly ($P < 0.01$) greater rate of salivation during rest than the bloater-type animals (Table 8); therefore, type appears to exert an influence on salivation, whether feeding a dry or succulent ration.

Rate of salivation during feeding shows significance for the variable cow-within-type ($P < 0.05$), whereas the variable for cows was significant at the 1% level. This indicates that variations among cows were sufficiently great as to make it questionable whether type was actually of significance in this case. The nonbloater, with a higher mean value and a relatively small standard error, suggests, on the other hand, that type may have some influence on salivary secretion, even on a hay diet. Not enough animals were used to clearly show whether a type effect exists in a larger population of cattle. Further studies must be conducted to clarify this point.

Salivary composition was quite variable on the hay diet, with only potassium being significant for type ($P < 0.05$). The reason for the wide variation in potassium values is not readily discernible; other variations appear to be normal fluctuations which one might expect to occur as the result of sampling error and normal biological variations.

Water consumption varied widely between cows, and individual consumption fluctuated markedly from day to day (Table 9). The higher rates for Cows 37 and 75, on the chopped hay ration, can be explained by the fact that twice during the early part of the study both cows lost their fistula plugs, with resulting loss of ingesta. When this occurred, water consumption increased approximately twofold.

An increase in water consumption accompanied increasing dryness of the feed (Table 9); however, differences in ambient temperatures tend to distort these data, i.e., the daytime temperatures during the alfalfa-tops trials ranged from approximately 90 to 108° F., in contrast to a daytime temperature range of 30 to 70° F. during the hay trials.

Conclusions: (a) Type (bloater or nonbloater) may exert a significant effect on salivary secretion during rest and during feeding. (b) Saliva potassium concentration was significantly higher ($P < 0.05$) in bloaters than nonbloaters; other salivary constituents did not differ significantly. (c) Dry matter content of the whole ingesta was somewhat lower in the nonbloater. (d) Daily water consumption was similar for both types.

DISCUSSION

Determination and proper classification of susceptibility to bloat is an indispensable, preliminary procedure to any investigation of the so-called animal factors.

Bloat susceptibility was well defined over a 2-yr. period in the animals of the current studies. Susceptibility, it was pointed out, is not absolute; rather, individuals exhibit a certain tendency to bloat. For example, an animal classified as a nonbloater may, on rare occasions, actually experience slight bloat. These temporary departures from the normal trend seemed to be of limited importance in the current studies; therefore, no attempt was made to reclassify an animal when it failed to respond, as during the classification period.

Rapid gas production is undoubtedly important in the production of bloat (14, 28). Fermentation gas, in the presence of certain viscous or foam-stabilizing materials, produces a frothy mass which the animal is unable to expel. Dougherty and Habel (17) have shown the cervical esophageal sphincter to be reflexly inhibited by foam or fluids but not by free gas. The inhibitory receptors were found to be located in a small area around the cardia (18), a finding which suggests that it would be impossible, or difficult at best, for a ruminant to eructate during bloat, because of the foaming mass of ingesta, except of course, during the terminal stages.

Inhibitory receptors do not appear to be important during legume bloat, however, because in cases of spontaneous recovery, the cardia apparently is well covered with foam when the first belches begin to occur. Colvin (13) has observed, in the face of increased eructation frequency, a marked retention of gas during the initial stages of bloat but later, as the foam began to break down, large volumes of gas were expelled with each belch. Johns *et al.* (25) also question whether inhibition of the cranial sphincter is important in bloat, since they were not able to observe, in bloated cows, the obvious swallowing of foam and liquid seen in calves and sheep prepared according to Dougherty *et al.* (18).

The role of saliva in the etiology of bloat has been considered by numerous workers (5, 8, 10, 25, 34, 36, 37, 43, 45), but at present is still poorly understood. Nevertheless, it is indicated that saliva may have a multiple influence on the development of bloat by (a) contributing to the production of gas (10), (b) dilution of the ingesta (45), (c) addition of foaming agents, namely mucoproteins (25), (d) alteration of rumen surface tension (25), and (e) other reactions yet unknown.

Saliva high in bicarbonate content and buffering capacity, and low in protein,

is continuously secreted by the parotid glands. Submaxillary saliva, on the other hand, is secreted only during feeding, is rich in protein compounds with strong foaming properties, and is low in buffering capacity. Sublingual saliva is intermediate in buffering capacity and protein content and is also secreted intermittently.

Kleiber (10) has calculated that gas production from parotid bicarbonate can amount to 120 liters CO_2 per day when being secreted at pH 8.2 and entering rumen contents of pH 6. Interestingly, the amount of CO_2 required to increase intrarumen pressure has been reported by Johns *et al.* (25) to be less at higher than at lower rumen pressures. These authors suggest that, under proper circumstances sufficient CO_2 may be produced from parotid bicarbonate to determine whether bloat is severe.

Only during rapidly developing bloat does gas from this source appear to be important, and then only if the pH is low, because cows can expel large volumes of gas during normal eructation and, with elevated intrarumen pressure, frequency of eructation contractions is known to increase (13). However, should gas become entrapped in foam, no belching of gas can occur and salivary CO_2 may then be important.

Phillipson and Reid (37) have found increased parotid and submaxillary flow in calves and sheep in response to increasing intrarumen pressures of 8 to 20 mm. Hg. Secretion response was variable, particularly in sheep, but usually parotid, submaxillary, and residual secretions increased in the calves; further increases in pressure inhibited salivary secretion.

Johns and associates (25) present data from Mangan which show that an increase of total nitrogen in pressure stimulated submaxillary saliva from 29.2 to 116.8 mg. N/100 ml. of saliva. This finding could be of signal importance in the etiology of bloat, as submaxillary mucoprotein is a strong foaming agent. Thus, during the early stages of bloat, if eating did not cease as intrarumen pressure increased, large amounts of submaxillary mucoproteins would be added to the already foaming ingesta, with more foam being produced and, perhaps, increased foam stability. However, physical properties of human saliva have been shown by Dewar and Parfitt (16) to be determined by the state of the mucin present and not by the actual concentration. The percentage of polymerized mucin (unhydrolyzed) determines *spinnbarkeit*. Thus, as salivary mucin enters the relatively more acid ingesta, considerable hydrolysis is likely to occur, with resulting reduction of viscosity and spinnbarkeit. If physical properties of bovine submaxillary saliva are similar to those of humans, submaxillary saliva may not be as great a source of foaming material as presumed. Obviously, much remains to be done before the role of submaxillary mucoproteins in bloat is known.

Weiss (44) found a thick, viscous ingesta in sheep fed succulent herbage and a thin, watery ingesta in sheep grazing coarse, dry forage. He concluded, on the basis of these findings, that dry feed stimulated salivary secretion, with resulting dilution of rumen ingesta, and decreased incidence of bloat as a consequence.

Conversely, dilution of the ingesta, Ferguson and Terry (19) suggest, is unimportant in bloat, because they were able to produce the condition by drenching with alfalfa juice in the presence of pilocarpine-induced saliva. Such treatment does not simulate conditions normally found during bloat. In fact, drenching seldom produces the condition unless an animal has first been fed bloatable herbage for a few days. In this respect, Boda (7) found that considerable fermentable substrate was necessary before he could produce satisfactory bloat when drenching cattle with egg white.

In view of the finding that rates of salivation were significantly greater in nonbloaters than in the bloaters, amount of rumen fluid appears to be important to bloat susceptibility. The nonbloaters were calculated to be capable of producing as much as 117 liters of mixed saliva per day, in contrast to a maximum secretion of 86 liters per day for the bloaters.

Nonbloater ingesta was characteristically thin and watery, whereas that of the bloaters was thick and viscous, with a high dry matter content, in spite of similar water intake (Table 9) and feed consumption (Table 6).

Dry matter content of prefeeding ingesta closely paralleled bloat. Four cows with thin, watery ingestas did not bloat during the salivary study, but two cows (80 and 84) bloated frequently and were consistently observed to have thick, viscous ingesta with high dry matter contents (Table 6). Barrentine (4) has reported a similar observation in bloating and nonbloating steers grazing alfalfa.

Rumen dry matter concentration can influence parotid secretion. Stewart and Dougherty (41) have shown that dilution of rumen contents with water results in reduced parotid flow with increased salivation following ingestion of hay. Rumen dry matter content per se is not likely to be indicative of its ability to stimulate receptors in the rumen. In the current studies, prefeeding ingesta from the bloaters fed alfalfa tops was characteristically a homogeneous mixture with no recognizable particles of forage remaining, whereas similar ingesta from these animals fed chopped hay still contained numerous short, stiff particles presumed to be capable of stimulating tactile receptors of the rumen, as reflected by an increased rate of salivation. Thus, the physical nature of the dry matter also appears to be of importance in stimulating salivary flow.

The relationship between bloat susceptibility and rate of salivation is not clear and is further confused by the fact that bloat rarely occurs the first time an animal consumes succulent legumes. If, however, continued grazing is permitted the incidence of bloat usually increases. This suggests that an important factor or factors accumulates in the prefeeding ingesta of animals fed such material. Reduced accumulation of these materials may occur in the dilute ingesta of nonbloaters as the result of (a) more complete forage digestion (fermentation end-products are less limiting in a dilute solution), (b) larger amounts of salts entering the rumens of nonbloaters daily and, therefore, greater total buffering capacity, with prolonged fermentation as a possible consequence, (c) gas release enhanced by larger amounts of mucin entering the rumens of nonbloaters, (d) lower ingesta surface tension brought about by a large volume

of parotid saliva, and (e) a combination of several or all of the above factors.

Hungate and associates (23) found a greater rate of fermentation in bloating than in nonbloating animals, which suggests that more available fermentable substrate was present in the prefeeding ingesta of bloating animals. Their data do not show this to be the case, yet the thick, viscous mass, with greater dry matter content found in the bloaters of the current study, support this hypothesis. Paradoxically, in the current trials, total gas production was found to be similar between the two types of animals. Perhaps the techniques employed were insufficiently sensitive to detect small but real differences in early fermentation rates or, because bloat was slight during this portion of the study, no differences could be expected.

With regard to mucin, Van Horn and Bartley (43) have shown salivary and plant mucin to be efficacious in enhancing gas release from frothing ingesta in vitro. Bartley's (5) in vivo studies were less conclusive, but do not exclude the possibility that saliva may reduce the probability of bloat.

The foam of bloat is of the surface viscous-type (25) and any agent which can displace the surface-active agents of this system tends to form a more stable thermodynamic state, with reduced foam stability resulting. Generally, those agents with low surface tensions are more surface-active than agents with high surface tensions. The former will displace the latter on the foam surface and in so doing produce a more stable thermodynamic state, and a return to the liquid form.

Surface tension of parotid saliva is relatively low (39) and owing to its continuous secretion may very well reduce, after several hours, rumen surface tension sufficiently to prevent stable foam formation at the time of feeding.

Since nonstable foam was commonly found over the ingesta of the nonbloaters following ingestion of alfalfa tops, we suggest that mixed saliva added to the rumen during periods of rest could prevent stable foam formation by reducing ingesta surface tension and by promoting more complete digestion of the forage, with fewer agents responsible for foam stabilization accumulating in the prefeeding ingesta as a result.

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EFFECT OF FEEDING SODIUM AND POTASSIUM BICARBONATE ON MILK FAT, RUMEN pH, AND VOLATILE FATTY ACID PRODUCTION[1]

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SUMMARY

Sodium or potassium bicarbonates (1 lb/day) were added to the rations of cows fed grain to appetite and 2 lb. of long or pelleted hay. These bicarbonates prevented the decline in milk fat percentage frequently associated with such rations. The pH of the rumen contents was increased, but the molar proportion of the acids was not appreciably altered.

The feeding of restricted roughage with high levels of concentrates has resulted in depressed milk fat associated with increased proportions of propionic acid relative to acetic acid in the rumen (10, 13). Balch *et al.* (1, 2) observed low ruminal pH values for long periods of time in cattle fed low-roughage rations and demonstrated that such rations elicit less saliva production. Saliva diversion has also resulted in a low ruminal pH (7). Reid *et al.* (9) theorized that sustained low ruminal pH favored establishment of a flora particularly adapted to propionic acid production. The present experiments were conducted to test the theory that maintenance of normal ruminal pH would result in normal fermentation products and normal milk fat percentages.

EXPERIMENTAL PROCEDURE

Experiment 1. Two groups of six Holsteins each were used in a double-reversal trial with three periods of 21 days each. Milk production and milk fat was determined for the last 12 days of each period, using daily milk weights and three-day composites for Babcock fat analysis. All cows were offered 24 lb. of a grain mixture composed of 77.5% ground corn, 20% soybean oil meal, 1% dicalcium phosphate, 1% calcium carbonate, 0.5% trace-mineralized salt plus supplemental vitamins A and D plus 2 lb. of alfalfa hay per day. One pound of sodium bicarbonate was mixed with the grain ration at the time of feeding for the treatment group and replaced 1 lb. of grain. Orts were removed and weighed at three-day intervals. Statistical analysis was performed as outlined by Snedecor [(11), page 421].

Experiment 2. Three groups of three cows each were used with a Latin-square design. The three treatments were 1 lb. sodium bicarbonate daily, 1 lb. potassium bicarbonate daily, and control. The periods consisted of 21 days each and milk and milk fat were determined for the last 14 days of each period. The

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rations were the same as in Experiment 1, except 2 lb. of commercial dehydrated alfalfa pellets replaced the 2 lb. of hay.

Experiment 3. Three mature, nonlactating Holstein cows equipped with rumen fistulas were fed rations of: (A) 12 lb. alfalfa hay and 8 lb. of the grain mixture given in Experiment 1; (B) 14 lb. grain plus 1 lb. dehydrated alfalfa pellets; (C) Treatment B plus 1 lb. sodium bicarbonate.

The cows were rotated during the three periods, so that each cow received each ration. Rumen samples were collected 3 hr. after feeding on the last two days of each 14-day period. Samples were centrifuged immediately and pH determined with the glass electrode in the supernatant liquid. Supernatant liquid was preserved by adding 1 ml. of 50% (V/V) H_2SO_4 to each 50 ml. and assayed for organic acids by the method of Wiseman and Irvin (14), excepting lactic acid, which was determined colorimetrically (3).

RESULTS AND DISCUSSION

Experiment 1. Feed intake totaled 9,406 lb. for the treatment periods, versus 9,773 lb. for the control periods. Milk bloat occurred in both groups, but seemed to be aggravated by the sodium bicarbonate. This might explain the difference in feed intake, since the cows usually stopped eating while bloated. No hay was refused and some hay was stolen from neighboring cows in spite of high manger dividers. Weight gains averaged 0.04 lb/day for the treatment periods and 0.02 lb/day for the control periods. The sodium bicarbonate increased the milk fat percentage 0.28 unit ($P < 0.01$), but decreased milk production 0.5 lb/day (Table 1). The decreased milk production was not consistent with Experiment 2.

Experiment 2. Feeding sodium or potassium bicarbonate yielded a higher percentage of milk fat ($P < 0.01$) and slightly more milk (Table 1). The differences between sodium and potassium were small and nonsignificant. The cows averaged 0.1 lb/day gain in body weight during the experiment and treatment had little or no effect. Mild bloat was observed 39 times in the cows fed sodium bicarbonate, 44 times in the cows fed potassium bicarbonate, and 20 times in the controls. It would seem that either sodium or potassium bicarbonate is capable of exerting a lipogenic effect. The potassium appeared somewhat less effective than sodium per unit weight, but this could be explained by less potassium being fed on a molar basis.

TABLE 1

Milk production and fat

Treatment	Experiment I [a]		Experiment II	
	Milk	Fat	Milk	Fat
	(lb/day)	*(%)*	*(lb/day)*	*(%)*
Control	28.0	3.4[b]	23.7	2.1[b, c]
Sodium bicarbonate	27.5	3.7[b]	24.2	2.9[b]
Potassium bicarbonate			24.9	2.6[c]

[a] Adjusted means.

[b, c] Figures with the same letter are significantly different at the 1% confidence level.

TABLE 2

Composition of rumen fluid

	Rations		
	Normal	High grain	Grain + $NaHCO_3$
pH	6.3[a]	5.8[a, b]	6.4[b]
NH_4-N (mg/100 ml.)	13.7	14.8	19.1
Volatile acids (u*M*/ml)	106.4	112.7	110.5
Acetic (molar per cent)	72.4	68.4	67.4
Propionic (molar per cent)	16.7	18.4	18.2
Butyric and higher (molar per cent)	10.9	13.1	14.4
Lactic acid (μg/ml)	1.3	1.4	0.7

[a, b] Figures with the same letter are significantly different at the 5% confidence level.

Experiment 3. Sodium bicarbonate feeding increased the rumen pH (Table 2), but had little or no effect on organic acid composition. The high grain ration increased the molar per cent of propionic acid, but this increase only approached significance. The failure to find a bigger difference might be explained by the short experimental periods. The outstanding result was the failure to obtain a decrease in the molar per cent propionic acid when sodium bicarbonate was fed. It appears that the mechanism of sodium bicarbonate in preserving normal fat content of milk must be sought elsewhere.

Lampila (4) and Lampila and Poijarvi (5) have reported that more total volatile acid is produced in the rumen at higher pH within the range of 5.0 to 6.8. The molar per cent propionic acid was slightly lowered in their work. Van Campen and Matrone (12) found more total volatile acids and more propionic acid when sodium and potassium bicarbonates were added to sheep rations. Matrone *et al.* (6) have reported that bicarbonates increased appetite and weight gained. The increased feed intake might account for some of the increased volatile acid productions. Nicholson *et al.* (8) reported similar increased growth and feed intake and ruminal volatile fatty acids in calves fed rations fortified with alkaline ash constituents. If volatile fatty acid production was increased in the work reported here, absorption must have been increased enough to mask the effect. Since McManus (7) observed a greater absorption of volatile fatty acids from the rumen at pH 6.9 than at pH 6.3, this conclusion seems tenable. More work will be required to clarify the mode of action. Blood studies might be helpful.

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NITROGEN METABOLISM IN DAIRY CATTLE. II. SOYBRAN FLAKES VERSUS OATS AS PRINCIPAL SOURCES OF DIGESTIBLE CARBOHYDRATES FOR NITROGEN UTILIZATION[1]

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SUMMARY

Nitrogen metabolism, feed intake, and milk production were studied using six lactating dairy cows in a 3 × 3 Latin-square design. Soybran flakes and soybean oil meal were compared to either (1) soybran flakes, ground shelled corn, and soybean oil meal or (2) ground whole oats, ground shelled corn, and soybean oil meal as the principal sources of digestible nutrients. The two soybran flake mixtures were also compared to an alfalfa hay and grain concentrate ration, using six dairy heifers. The percentages of cellulose, starch, pentosans, polyuronides, and sugars were determined for each ration. Apparent protein digestibility reflected differences in the protein content of the complete rations. Cellulose digestibility was unusually high in the soybran flake rations, 77 to 79%. Higher average yields of milk nitrogen and larger positive nitrogen balances resulted in greater nitrogen efficiency in cows fed soybran flakes. However, differences in digestible dry matter intake were correlated with the differences in nitrogen utilization, and adjusting for digestible dry matter intake accounted for the differences in nitrogen efficiency. Abrupt shifts to high levels of cereal grain in the ration depressed milk yield because of lowered feed intake. It was concluded that cellulose is equal to starch for supporting nitrogen metabolism in growing or lactating dairy cattle, provided the cellulose can be digested.

This study is concerned with the efficiency of nitrogen utilization in lactating cows when the principal feed sources of digestible energy were either cellulose and hemicelluloses of soybran flakes or starch of ground whole oats.

In a previous study, it was shown that the nitrogen from freshly cut forages was utilized more efficiently by dairy cows than nitrogen from ensiled forages. Differences occurred primarily at high levels of protein equivalent in the ration, since below 15% protein equivalent in the ration, silage and freshly cut forage nitrogen were utilized with the same efficiency. But, utilization of silage nitrogen for milk and body retention declined in efficiency at higher levels of protein equivalent; whereas, freshly cut forage nitrogen was used efficiently at all levels of protein equivalent measured up to 24.6% (5). Head (11) has made similar observations in England, but found that most of the extra nitrogen utilized went for body retention. Adding cereal grain to forage rations increased the efficiency of nitrogen utilization markedly (5, 10). Holdaway *et al.* (13) showed that nitrogen efficiency decreased sharply when high levels of protein supplement were included in the grain concentrate.

El-Shazly (9), Cuthbertson and Chalmers (8), and McDonald (18) have demonstrated, using sheep, that efficiency of protein utilization decreases with

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increased solubility of the protein in the ruminal contents. A major route of nitrogen loss arose from the passage of ammonia directly across the rumen wall (8, 19). Because the nonprotein nitrogen of ensiled forage was found to be three or four times the amount contained in freshly cut forage, it was concluded that an increase in relative content of soluble nitrogen and a simultaneous decrease in soluble carbohydrate during fermentation caused the reduction in efficiency of nitrogen utilization below that obtained with freshly cut forage (4).

To obtain specific information on the significance of forage-type plant carbohydrates to nitrogen utilization in dairy cattle, it was thought advisable to simulate in a ration from natural sources carbohydrates found in freshly cut forage, and to determine their ability to support nitrogen utilization in lactating cows in comparison with the carbohydrates of cereal grain.

EXPERIMENTAL PROCEDURE

Soybean oil meal containing 50% protein was used as the major source of nitrogen. The carbohydrates were furnished from soybran flakes or ground whole oats, ground shelled corn, and molasses. Vtamin A and D supplement, defluorinated rock phosphate, salt and trace minerals were included in each ration. The formulae for two soybran flake mixtures and the oats mixture are shown in Table 1. The rations were formulated to be either high or very high in protein

TABLE 1

Ingredients and chemical composition of experimental concentrate mixtures

	Mixture 1	Mixture 2	Mixture 3	Mixed hay
	(%)			
	Ingredients [a]			
Soybran flakes	62.7	62.7	.....	.....
Ground whole oats	.....	.....	62.7	.....
Ground shelled corn	.....	16.7	16.7	.....
Soybean oil meal	33.3	16.6	16.6	.....
Molasses	3.0	3.0	3.0	.....
Salt	0.5	0.5	0.5	.....
Defluorinated rock phosphate	0.5	0.5	0.5	.....
	Average chemical analysis			
Dry matter	86.3	86.5	87.2	84.3
Organic matter	79.3	81.0	82.2	76.6
Crude protein	25.4	18.1	24.3	10.4
Cellulose	26.5	29.2	8.5	34.2
Pentosans	12.0	12.5	6.0	19.4
Polyuronides	10.6	10.1	0.3	6.2
Fat	2.25	2.63	3.92	0.89
Ash	7.0	5.5	5.0	7.7
Starch	1.2	12.0	40.1	.....

[a] Micro-ingredients included the trace minerals iodine, copper, iron, zinc, manganese, and cobalt. One thousand I.U. of vitamin A and 500 I.U. of vitamin D were added per pound of mixture.

content, so that an excess of protein was available and so that two levels of protein were used with soybran flakes. The protein content of the very high protein soybran flake ration, Mixture 1—Table 1, was raised by substituting

soybean oil meal for the ground shelled corn. Likewise, Mixture 3, the control ration, was made by substituting ground whole oats for the soybran flakes, which made the protein content of Mixture 3 approximately equal to Mixture 1, Table 1.

These mixtures were fed to appetite to six cows with a limited amount of low-quality, grass-legume hay cut on June 19. The six milking cows were fed the various rations in accordance with a Latin-square experimental design, using sequences described by Cochran and Cox (3), for estimating residual effects. Separate collections of feces and urine (4) were made during the third week of each 3-wk. feeding period. Feed intake, feed refused, and milk produced were measured daily.

Dry matter, total nitrogen, ether extract, and ash were determined by conventional procedures (24). Cellulose was determined by the procedure of Crampton and Maynard (6). Polyuronides were estimated as the galacturonic acid equivalent of carbon dioxide produced from boiling 1.5 hr. with 19% hydrochloric acid at 145° C. (17). Pentosans were determined colorimetrically (1), using a perchloric acid extract of finely ground plant tissue (21).

In another experiment using two 6-8-mo.-old dairy heifers soybran flake Mixtures 1 and 2 were fed with mixed hay and compared to an alfalfa hay–grain concentrate ration. Digestibility and nitrogen utilization were measured. The grain concentrate was composed of 17% soybean oil meal, 50% ground shelled corn, 21% ground whole oats, 1% iodized salt, and 1% steamed bonemeal.

RESULTS

Chemical analyses of samples taken from the various feeds eaten by the experimental cows showed the protein content to be: Ration 1, 24.7%; Ration 2, 17.6%; and Ration 3, 21.2% (Table 2). Distribution of the major component

TABLE 2

Protein and carbohydrate composition of complete rations consumed [a] (Dry basis)

	Ration 1	Ration 2	Ration 3
		(%)	
Protein	24.7	17.6	21.2
Cellulose	32.7	34.7	22.8
Pentosans	15.9	16.4	14.1
Polyuronides	11.2	10.9	3.4
Starch	.....	9.1[a]	23.2[a]
Sugars	1.5[a]	1.5[a]	1.8[a]

[a] Starch and sugar values calculated and based on percentages of cereal grain in the ration and average values taken from tables on composition of cereal grains (22).

carbohydrates in the various rations are also shown. The major carbohydrates varied from 32.7% cellulose and no starch in Ration 1 to 22.8% cellulose and 23.2% starch in Ration 3. The total pentosan content was approximately the same for each ration, but polyuronide content was three times higher in the soybran flake rations than in the oats ration.

TABLE 3

Comparison of digestibility of rations, efficiency of nitrogen utilization, feed intake, and milk production by cows when either soybran flakes or ground whole oats provided the major carbohydrate components of the ration and were fed with mixed hay

	Complete Experimental Rations			
	Ration 1	Ration 2	Ration 3	L.S.D. $P < 0.05$
Dry matter digested *(%)*	76.6	75.3	69.3	5.7
Cellulose digested *(%)*	78.9	76.6	55.8	10.1
Total digestible nutrients *(%)*	72.1	71.9	66.7	.....
Digestible dry matter/1,000 lb. B.W. *(lb/day)*	21.7	22.9	19.0	3.1
Experimental ration consumed *(lb/day)*	29.2	30.3	20.2	.....
Mixed hay consumed *(lb/day)*	9.1	9.2	15.8	.....
Apparent protein digestion *(%)*	78.4	72.5	77.9	4.7
Nitrogen utilization				
1. Nitrogen intake *(g/day)*	596	450	482	.....
2. Apparent nitrogen absorbed *(g/day)*	467	326	375	.....
3. Fecal nitrogen *(g/day)*	129	124	107	.....
4. Urine nitrogen *(g/day)*	291	168	234	.....
5. Milk nitrogen *(g/day)*	106	100	91	N.S.
6. Nitrogen balance *(g/day)*	70	58	50	N.S.
7. Total nitrogen used *(g/day)*	176	158	141	N.S.
Nitrogen efficiency *(%)*[a]	29.1	35.0	28.3	6.1
Nitrogen efficiency adjusted *(%)*[b]	28.2	33.4	30.8	2.4
$\frac{\text{Milk N}}{\text{Absorbed N}}$ *(%)*	22.7	30.7	24.2	2.8
Urine nitrogen *(%)*	48.8	37.3	48.6	8.9
Milk *(lb/day)*	47.1	46.0	43.1	3.9
4% F.C.M. *(lb/day)*	41.8	41.2	40.3	N.S.
Milk protein *(%)*	3.08	3.04	2.89	N.S.

[a] Per cent of intake used for milk protein and nitrogen retention.
[b] Nitrogen efficiency adjusted for differences in digestible dry matter intake by analysis of covariance.

Results of digestion and nitrogen balance trials are summarized (Table 3). Although all rations were offered free-choice, the cows ate less experimental mixture and more hay when fed Mixture 3 than when Mixture 1 or 2 was fed. Attempts to raise the level of grain consumption in cows fed Mixture 3, by limiting the amount of hay fed, resulted in increased amounts of refused grain and in one animal becoming bloated. Consequently, the average total protein content of Ration 3 was lower than had been anticipated, because of the low protein content of the extra hay used.

Apparent protein digestibility did not differ markedly among the complete rations, but reflected the differences in the protein content of Rations 1 and 2 (Table 3). Cellulose digestibility was unusually high in the soybran flake–mixed hay ration and both dry matter and cellulose digestibility were significantly less during the periods when oats and mixed hay were fed ($P < 0.01$).

Comparisons of the nitrogen efficiency values indicated the effective usage of nitrogen in cows fed soybran flake rations (Table 3). The per cent protein content of the ration and nitrogen efficiency percentages deviated from the theoretically expected inverse relationship, suggesting a direct ration effect. Nitrogen efficiency percentages were 29.1, 35.0, and 28.3, respectively, for Ra-

tions 1, 2, and 3; whereas, the protein contents were 24.7, 17.6, and 21.2. However, associated with the lower protein anabolism in cows fed the oats–mixed hay ration was a lower digestible dry matter intake. That differences in feed consumption were partly responsible for the differences in nitrogen utilization was suggested by the positive correlation coefficient, $r = 0.554$, between digestible dry matter intake and the amount of nitrogen used. When the mean nitrogen efficiency percentage of each ration was adjusted by analysis of covariance (23), for the observed differences in digestible dry matter intake, the adjusted means in contrast to the true means were found to be inversely related to the per cent protein content of the rations and each differed significantly from the other. Milk production was highest during the period of soybran flake feeding. There were no notable differences among the groups in the amount of 4% fat-corrected milk produced. Milk protein showed a slight average decline but was not significant (Table 3).

The results with heifers showed no marked differences in nitrogen retention between soybran flake rations and the alfalfa hay–grain concentrate ration (Table 4).

TABLE 4

Digestibility and nitrogen utilization in dairy heifers fed rations high in soybran flakes compared with heifers fed alfalfa hay and grain concentrate

	Soybran flakes Ration 1	Soybran flakes Ration 2	Alfalfa hay and grain
No. of animals	2	2	2
Av. body weight *(lb.)*	464	445	404
Dry matter digestibility *(%)*	73.4	74.8	72.4
Protein digestibility *(%)*	77.8	71.5	74.6
Nitrogen balance *(g/day/100 lb.)*	16.9	11.0	12.5
Nitrogen efficiency *(%)*	44.6	40.1	34.6
Feed intake			
Experimental mixture *(lb/day)*	7.5	9.2	.....
Mixed hay *(lb/day)*	2.3	1.6	.....
Alfalfa hay *(lb/day)*	.....	.....	9.7
Grain concentrate *(lb/day)*	.....	.....	4.0

DISCUSSION OF RESULTS

Rations consisting of soybran flakes, soybean oil meal, a small amount of ground corn and molasses, and limited hay effectively supported nitrogen utilization in dairy cows; whereas, substituting ground whole oats for the soybran flakes reduced nitrogen utilization. This was true even though the cellulose content of the soybran flake ration was higher than percentages usually found in a typical forage-grain type ration. For example, a ration consisting of 20% grain and 80% alfalfa-grass forage contained 20% protein, 24% cellulose, 12% polyuronides, 5% pentosans, 6% starch, and 3% sugars, compared to Ration 2, Table 2, which contained 18% protein, 30% cellulose, 9% polyuronides, 14% pentosans, 6% starch, and 1.3% sugars.

Convincingly demonstrated by the results is the principle that the carbohydrates in a ration, largely through effects on feed intake, effectively alter the

efficiency of nitrogen utilization and determine the optimum amount of nitrogen that may be used efficiently in a ration. Moreover, the results form a basis for postulating that cellulose, pentosns, and polyuronides are useful for efficient utilization of the protein contained in soybean oil meal. In this regard, Bryant and Robinson (2) have shown that certain cellulose-digesting rumen microorganisms require ammonia nitrogen and thus possibly serve as nitrogen conservers in the rumen.

Cunningham *et al.* (7) raised the protein content of the ration of young calves to 24% of the total calories without diminishing the efficiency of nitrogen utilization by simultaneously increasing the energy intake. Hibbs and Conrad (12) found that high-roughage pellets with a nutritive ratio of 1 : 3.9 supported higher body weight gains in young calves than similar rations with wider nutritive ratios. The high nitrogen efficiency found in dairy cows fed freshly cut forage high in protein was discussed in the introduction to this paper (5). In lactating cows, Logan *et al.* (15) found that cows fed a high-protein, high-energy ration produced more milk and gain in body weight than control cows fed a normal protein high-energy ration, provided they also consumed more digestible nutrients daily. Because of these results, and the results of this experiment, it is postulated that many sources of nitrogen fed to dairy cattle could be used efficiently up to 18% total protein in the ration if the carbohydrate mixtures which would support maximum digestible dry matter intake were always used.

The reduced yield in milk of cows fed the oats and mixed hay ration has been noted in the results. The question may be raised as to whether this represents a temporary condition effected by the abrupt shift to a high level of starch and more hay in the diet, which depressed ruminal cellulose digestion and feed intake. That the conditions of lowered milk yields and nitrogen utilization are temporary is suggested by the finding that suboptimum digestible dry matter intake occurred simultaneously. Because of the present emphasis on high-grain feeding in certain areas, a more expeditious experimental design involving total lactation studies is needed to clarify this effect.

The observations made on digestibility of soybran flakes are in agreement with the results obtained by Quicke *et al.* (22) and Loosli (16).

It appears that optimum nitrogen retention was obtained in growing heifers using the ration high in cellulose and devoid of starch, but that the cellulose fraction was known to be unusually high in digestibility. Thus, it is concluded that cellulose is equal to starch for supporting nitrogen metabolism, provided the cellulose can be digested. This conclusion is in line with the findings reported by Kellner (14).

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VALUE OF CORN DISTILLERS DRIED GRAINS, SOYBEAN OIL MEAL, HEATED SOYBEANS, AND SOYBEAN OIL MEAL PLUS STARCH FOR MILK PRODUCTION

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SUMMARY

Corn distillers dried grains were compared with different soybean products in concentrate mixtures for milking cows. Solvent extracted soybean oil meal and corn distillers dried grains with solubles were approximately equal in value when they supplied the primary supplemental protein in a concentrate mixture. When heated soybeans, containing all of the fat, were used to furnish an equal amount of protein, cows produced 2.5 lb. more milk daily and the milk was 0.14 percentage unit lower in fat content than with solvent extracted soybean oil meal. The addition of purified starch to the concentrate in an amount to furnish energy equal to the fat in the heated soybeans had no significant effect on milk yield or the fat percentage of the milk.

Soybean oil meal has been used extensively in dairy rations for several decades with satisfactory results. Raw soybeans have also been fed to cattle in limited amounts to supply protein and fat (11). Many studies with ruminants, especially those involving the use of nonprotein nitrogen, have led to the presently accepted view that all sources of crude protein are approximately equal for cattle and sheep. Differences in the fat content of various high-protein feeds have been shown to influence their value for milking cows (12), but relatively few recent studies have compared the different protein sources for milk production. Corn distillers dried grains as the supplemental protein source in concentrate mixtures for milking cows resulted in higher milk yields than other high-protein feeds having lower fat and energy contents. Of the several protein feeds tested, only soybean oil meal appeared to be equal in value to distillers grains (7, 9). This result seemed surprising, since the solvent extracted soybean oil meal studied was very low in fat content and is rated lower in TDN content in standard feed tables. Further comparisons with soybean products seemed desirable to extend the observations.

EXPERIMENTAL PROCEDURES

A 4 × 4 Latin-square experiment was carried out to compare the concentrate mixtures described in Table 1. Three of the concentrates provided a comparison of corn distillers dried grains with solvent extracted soybean oil meal and a heated soybean product containing all of the natural soybean oil, and the fourth contained corn starch to furnish energy equal to the fat in the soybeans.

The chemical analyses of the feeds used are summarized in Table 2.

The cows were fed timothy-grass mixed hay and corn silage in addition to the concentrates. The roughages were fed in amounts such that all cows left

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TABLE 1
Composition of the concentrate mixtures

Ingredients	Mixture A	E	F	G
	(lb.)			
Ear corn, ground	800	1,000	1,100	800
Oats, ground	200	200	200	200
Corn distillers dried grains with solubles	800	.....	.....	.....
Heated soybeans	.....	600	.....	.....
Soybean oil meal, solvent extracted	.....	.....	500	550
Corn starch	.....	.....	.....	250
Cane molasses	160	160	160	160
Dicalcium phosphate	20	20	20	20
Salt	20	20	20	20
Total[a]	2,000	2,000	2,000	2,000

[a] Two grams of cobalt sulfate were added per ton.

1 to 3 lb. per day, and records were made of the amounts consumed. The concentrates were fed at levels estimated to furnish TDN equal to the NRC requirements at the start of each period and no changes were made during the 6-wk. period.

Sixteen purebred Holstein cows were arranged into four replicates of four cows each on the basis of age and level of production during the preliminary period. Assignment to the four concentrates was made at random within replicates. One replicate consisted of mature cows, one was made up of second-calf heifers, and the others were in their first lactations. All cows were observed for a period of 6 to 8 wk. to standardize feed intakes and to evaluate production before they were assigned to the dietary treatments. All cows had passed their peaks of production, or had been in milk for 8 wk. and they had not been bred more than 2 wk. at the start of the test.

RESULTS AND DISCUSSION

The average milk and fat production on the four concentrate mixtures is summarized in Table 3.

Average daily milk yield was significantly higher on heated soybeans than on the other mixtures and the fat percentage of the milk was significantly lower.

TABLE 2
Chemical composition of the feeds

	Moisture	Protein	Fat	Fiber	NFE	Ash
	(%)					
Concentrate mixtures containing:						
Corn distillers dried grains with solubles	11.5	16.0	5.3	10.5	52.1	4.6
Heated soybeans	15.8	18.5	5.3	8.2	47.8	4.4
Soybean oil meal	13.4	17.9	2.6	9.3	52.3	4.5
Soybean oil meal plus starch	16.8	19.0	1.4	7.5	50.8	4.5
Corn silage	69.6	2.4	0.7	9.2	16.7	1.4
Mixed hay[a]	6.4	8.2	1.7	33.5	45.0	5.2

[a] U. S. No. 2 timothy, light grass mixed hay.

TABLE 3

Average daily milk production, fat percentage, fat, FCM, and weights

Concentrate containing	Milk	Fat	Fat	FCM	Body weight	Weight gain
	(lb.)	*(%)*	*(lb.)*			
Corn distillers dried grains with solubles	40.9	3.63	1.48	38.6	1,160	.79
Heated soybeans	43.4**	3.49**	1.51	40.0	1,158	.61
Soybean oil meal	40.4	3.66	1.48	38.3	1,170	.92
Soybean oil meal plus starch	40.8	3.58	1.46	38.3	1,164	.64

** Significantly different ($P < 0.01$).

Average yields of fat and FCM were slightly higher than the others, but not significantly so.

The higher milk yield on the ration containing heated soybeans may be expected to have resulted from the higher energy value contributed by their higher fat content. Digestion studies with four steers yielded the following digestion coefficients for the heated soybeans: protein 87%, ether extract 94%, crude fiber 57%, and nitrogen-free extract 81%. The digestible protein content was 32.9% and the total digestible nutrient (TDN) value was 100.0%. For raw soybeans Morrison (13) lists digestion coefficients of 89% for protein, 88 for fat, 0 for fiber, and 67% for NFE. This gives a digestible protein value of 33.7% and a TDN value of 87.6%. Only for NFE was the digestibility substantially higher in the heated soybeans than in the raw seeds.

The depressed fat percentage on the heated-soybean ration is of special interest, since raw soybeans increase the fat test (4, 10). Ensor *et al.* (3) have reported that waste bread and other heated cereal grains depressed the fat percentage of the milk of cows because of a lowered acetate content of the rumen ingesta. Earlier reports by Balch *et al.* (1) had shown depressed fat percentage of milk of dairy cattle when flaked corn (heated) was fed. Feeding restricted amounts of hay or other fibrous, coarse feed will also depress the fat percentage of cow's milk by lowering the acetate content of the rumen contents, and feeding sodium acetate will overcome the lowered fat percentage (14). In none of these studies, however, was an increased milk yield associated with the depressed fat test.

The addition of corn starch to equal the energy in the fat of the heated soybeans had no measurable influence on milk yields or the fat content of the milk. In some studies starch has resulted in a lowered digestibility of feeds (2), but with legume hay no depression was noted (5). Although digestibility of the concentrates was not measured in our studies, it seems unlikely that there was any important depression, since milk yield was not lowered. In this study, as in a previous one (7), the concentrate containing soybean oil meal and having only 2.6% of fat by analyses produced as much milk and fat as the concentrate containing corn distillers dried grains with solubles and analyzing 5.3% in fat content. Likewise, the yield of milk and fat was equally high on the mixture having soybean oil meal and purified corn starch and containing only 1.4% of fat. These results are clearly not in agreement with earlier studies at Cornell

(8, 11, 12), in which extra fat in dairy feeds always stimulated milk production. While no satisfactory reason for the different response is evident at this time, the possible importance of protein quality or availability for high-producing cows seems worthy of study.

TABLE 4

Average daily feed and TDN intakes, and FCM per pound of TDN

Concentrate containing	Daily intakes				FCM per lb. TDN
	Hay	Silage	Concentrate	TDN [a]	
	(lb.)				
Corn distillers dried grains with solubles	11.5	34.7	14.8	23.8	2.97
Heated soybeans	11.5	35.4	14.8	24.6	2.78
Soybean oil meal	12.0	37.0	14.3	23.8	3.04
Soybean oil meal plus starch	12.1	35.6	14.6	24.1	2.78

[a] Estimated from Morrison's tables (13), except for heated soybeans, where the measured value of 100 was used.

The TDN intakes in Table 4 were estimated by using Morrison's (13) digestion coefficients for individual feeds. Values of 52% TDN for the hay, 20% for the corn silage, and 73.4, 77.9, 71.1, and 73.0% TDN for the concentrate mixtures A, E, F, and G, respectively, were arrived at. After allowing for the TDN used for maintenance and body gain, the pounds of FCM produced per pound of residual TDN consumed were calculated. It was assumed that each pound of weight gain required 3.53 lb. of TDN (6) and that the maintenance requirements were 8.0 lb. of TDN for the cows in all treatment groups. As an average, slightly less FCM was produced per pound of TDN on the concentrates containing heated soybeans and soybean oil meal plus starch than on the others.

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EFFECT OF THE ADDITION OF CITROLAS TO HIGH-MOISTURE FORAGE FOR SILAGE ON NUTRIENT LOSSES AND ANIMAL RESPONSE

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SUMMARY

Four tower silos were filled with moderately high-moisture oat forage with the addition of (a) 0, (b) 149, (c) 274, and (d) 425 lb. of citrolas crumbles per ton of fresh forage. Total dry matter losses calculated as a percentage of the forage dry matter ensiled were (a) 14, (b) 14, (c) 24, and (d) 31, respectively. Corresponding dry matter losses by seepage were: (a) 5.3, (b) 7.3, (c) 2.7, and (d) 1.7. Thus, with the lowest level of citrolas, seepage dry matter losses were higher than when none was added. The fermentation losses were much higher in the two silos with the most additive. The dry matter content and specific gravity of the seepage were higher with the additive, but the amount of additive had no effect. The maximum average temperatures were not affected by the treatments. Each of the silages was fed to six lactating dairy cows in a continuous-type feeding experiment. There was no significant difference in weight gains, milk production, or fat test of the milk. Cows fed the silages containing the two lowest amounts of the additive consumed highly significantly more silage dry matter than did those fed the other silages. Average pounds of silage dry matter consumed per thousand pounds of body weight were (a) 18.6, (b) 22.1, (c) 21.0, and (d) 19.0, for the four groups, respectively.

In recent years, farmers have become increasingly aware of the higher nutritive value of grasses at immature stages of growth. Because of this, and the prevalent practice of harvesting forages without wilting, a considerable amount of high-moisture forage is used for silage. Ensiling such material without additive frequently results in excessive losses by seepage and an undesirable fermentation. To overcome these adverse effects, various additives are often used and recommended. It is generally agreed that such materials should supply readily fermentable carbohydrates and have a high moisture-holding capacity. With these ideas in mind, a product known as citrolas crumbles was developed for use in making grass silage.

The purpose of this study was to determine the effects of the addition of various levels of the citrolas crumbles to high-moisture forages on the nutrient losses and silage quality. Since it was shown earlier (3) that the addition of citrus pulp apparently increased the dry matter content of seepage, and since citrus molasses are very soluble, it seemed especially important to determine the influence of various levels of the citrolas on seepage composition and total losses by this process.

EXPERIMENTAL PROCEDURE

Four tower silos measuring 8 by 24 ft. were filled with moderately high moisture forage, with the addition of (a) 0, (b) 149, (c) 274, and (d) 425 lb.

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citrolas crumbles per ton of fresh forage. The crumbles were a blend of citrus molasses, 33%, citrus meal, 20%, citrus fines, 37%, and citrus pulp, 10%. After blending, the material was pelleted and then crumbled. A forage mixture containing 95% oats in the flower stage of maturity, 3% crimson clover, and 2% other plants was ensiled April 28-30, 1959. The forage, which yielded 12.2 tons of fresh weight and 2.2 tons of dry matter per acre, was harvested with a conventional cutter-bar type forage chopper, using a ¼-in. theoretical cut. Each load of forage was weighed and sampled for chemical analyses by an accepted procedure (1). The citrolas crumbles were poured into the blower with the forage.

The seepage was sampled several times per day, as soon as it began flowing, with the subsequent frequency of sampling being approximately proportional to the rate of flow. All of the seepage was collected in barrels and measured. Temperature measurements were taken with thermocouples placed at eight to ten comparable locations in each silo, with all of the points being at least 2 ft. from the top, bottom, and sides.

Immediately after filling, the silos were sealed with green polyvinyl covers of 8-mil thickness, and a layer of sawdust about 4-6 in. deep was placed on top. Apparently, the sawdust prevented the accumulation of moisture just under the plastic, which has so often been mentioned as a problem when using this type of cover. When the silos were opened and the feeding experiment begun June 11, 1959, no top spoilage was observed. Silage samples were taken and chemical analyses made by procedures as described in a previous paper (3). In addition, specific gravity measurements were made on the seepage with a hydrometer. Total losses were determined by the difference between the amount of the nutrient put into the silo and the quantity removed.

Sixteen Holsteins and eight Jersey lactating cows were divided into four equalized groups. These were group-fed the silages ad libitum for 3 hr. twice per day, as the only source of roughage in a continuous-type feeding experiment which contained a pre-experimental standardization period of 12 days—plus a 53-day treatment period. One pound of concentrates was fed per day for each 5 lb. of 4% FCM produced during the preliminary period. The concentrate mixture used was composed of the following: ground snapped corn, 700 lb.; citrus pulp, 200 lb.; soybean oil meal, 100 lb.; salt, 20 lb.; and defluorinated rock phosphate, 15 lb. On a dry matter basis it contained: 13.1% crude protein; 11.2% crude fiber; 4.7% ether extract; and 5.6% ash.

Milk weights were taken and samples composited for milk fat analyses, using five-day periods. Body weights were taken the eighth, ninth, and tenth days, and the last three days of the experimental feeding period. This procedure has been shown to give more meaningful weight changes than taking the initial weights before the cows have become adjusted to the experimental feeds (3).

RESULTS AND DISCUSSION

The chemical composition data of the silages, forages, and the calculated values for the forage plus citrolas crumbles are presented in Table 1. Except

TABLE 1

Chemical composition of the forages, ensiled mixtures, and silages

	Control	Normal-level citrolas	High-level citrolas	Very high-level citrolas
Dry matter *(% of total wt.)*				
Forage	18.9	17.6	18.1	17.6
Forage + citrolas [a]		22.5	26.7	30.2
Silage	20.5	23.2	26.0	28.5
Crude protein *(% of dry matter)*				
Forage	10.1	11.0	10.6	10.4
Forage + citrolas [a]		9.8	9.0	8.5
Silage	9.4	9.9	9.2	9.3
Ether extract *(% of dry matter)*				
Forage	3.1	3.1	2.8	3.2
Forage + citrolas [a]		3.0	2.8	3.0
Silage	3.1	3.6	3.6	3.6
Crude fiber *(% of dry matter)*				
Forage	33.3	31.1	31.4	31.1
Forage + citrolas [a]		26.6	24.7	22.6
Silage	38.5	30.5	28.5	24.6
N.F.E. *(% of dry matter)*				
Forage	46.5	47.2	48.2	47.8
Forage + citrolas [a]		52.5	55.5	57.5
Silage	42.6	48.3	50.8	53.9
Ash *(% of dry matter)*				
Forage	7.0	7.6	7.1	7.4
Forage + citrolas [a]		8.0	8.0	8.4
Silage	6.4	7.7	7.8	8.5
pH of silage	4.5	4.7	4.5	4.7

[a] Calculated from analyses of forage and citrolas.

for differences related to dilution by the citrolas crumbles, most of the differences among silages appear to be minor.

The losses during the ensiling period are presented in Table 2. Dry matter losses were calculated both on a total basis and as a percentage of the forage dry matter. As discussed in a previous paper, calculating losses as a percentage of the forage material appears to be a more practical basis (3). While it is recognized that some of the additive nutrients would be lost during ensiling, it is also apparent that if the feed additive had not been put in the silo it would all have been available for feeding. As a percentage of the total dry matter ensiled, the invisible dry matter losses were 8.5, 5.0, 12.8, and 14.1, respectively, for the silos, in order of the increasing amounts of citrolas added. Thus, an important factor affecting the invisible dry matter loss was the very large amount of dry matter added by the citrolas. The total dry matter loss, when expressed as a percentage of forage dry matter, was approximately the same for the control and the silo to which a normal level of citrolas crumbles was added. With the high and very high levels of citrolas, the total dry matter loss increased considerably.

The dry matter loss by seepage was 38% higher from the silo which had the normal level of citrolas crumbles added than from the control. At the high and very high levels of citrolas crumbles the dry matter loss by seepage decreased.

TABLE 2

Ensiling losses as measured by in-put out-put data

	Control	Normal-level citrolas	High-level citrolas	Very high-level citrolas
Total *(% of total forage wt.)*				
Seepage	15.1	13.5	5.6	3.4
Invisible	5.1	0.4	8.2	8.4
Total	20.2	13.9	13.8	11.8
Dry matter *(% of total d.m.[a])*				
Total	13.8	10.3	14.4	14.9
Dry matter *(% of forage d.m.)*				
Seepage	5.3	7.3	2.7	1.7
Invisible	8.5	6.9	21.4	29.3
Total	13.8	14.2	24.1	31.0
Crude protein *(% of forage d.m.)*				
Seepage	10.9	8.8	3.7	2.0
Invisible	9.0	2.9	14.2	8.9
Total	19.9	11.7	17.9	10.9
Crude fiber *(% of forage d.m.)*	0.5	+3.2	1.4	11.2
N.F.E. *(% of forage d.m.)*	21.0	26.9	41.8	50.5
Ash *(% of forage d.m.)*	20.5	19.6	29.7	31.0

[a] d.m. = Dry matter.

However, even at the very high level this loss was almost one-third as large as when no additive was used. The addition of the citrolas elevated the dry matter of the ensiled mixture considerably (Table 1). The very high level of citrolas plus forage as ensiled contained 30% dry matter compared to only 19% for the control. However, the total amount of seepage lost on a fresh basis only decreased from 15 to 3%. Thus, it would appear that the forage dry matter was much more effective in retaining moisture than was that in the citrolas crumbles. This also is reflected in the wide differences in the dry matter content of the silages.

Since there was no spoilage, the losses, other than seepage, may be termed the invisible or fermentation portion. Calculated as a percentage of forage dry matter, losses by this route were 8.5 in the control silage and only slightly lower when the normal level of citrolas was added. With the high and very high levels of citrolas, the invisible dry matter losses increased to 21 and 29%, respectively. These values are approximately three and four times that obtained from the silo with the normal level of the additive.

The nitrogen content of the seepage dry matter from the silos with the additive was much lower than that from the control silo (Table 3). Perhaps this is largely due to the dilution of the forage seepage dry matter with a material which was low in nitrogen. The dry matter and specific gravity of the seepage from the silos with the citrolas were appreciably higher than those from the control. It is interesting that the amount of dry matter in seepage from the silo with the normal level of citrolas was about as high as that from the silos with higher levels. The specific gravity, nitrogen, and dry matter content of the seepage from all the silos which contained any citrolas were about the same, thus suggesting that the lowest level may have been sufficient to satu-

TABLE 3

Chemical composition of the seepages

	Control	Normal-level citrolas	High-level citrolas	Very high-level citrolas
Dry matter *(%)*	6.7	9.5	9.2	9.5
N × 6.25 *(% of seepage dry matter)*	21.6	13.8	14.7	12.4
pH	4.7	5.3	4.7	5.3
Specific gravity	1.031	1.043	1.045	1.046

rate the liquid. Laboratory tests indicated that approximately 45% of the dry matter in the citrolas was soluble in water at pH's over the range of the silage seepage collected throughout the study. Thus, 20 lb. of the citrolas would contain enough soluble material to elevate the dry matter content of the seepage from the level observed in the control silo to that obtained when citrolas was added at the various levels. The specific gravity of seepage samples which contained more dry matter was higher than that of the seepage with less dry matter. This was true of samples taken from the same silo as well as between silos. The correlation value between these two measures for the combined data was 0.96, which suggests that specific gravity might be used as a method of determining dry matter content of silage seepage.

There was very little difference in the temperatures which developed in the four silos, with the maximum averages at any one time ranging from 91 to 94° F. for the various silos.

In Table 4, the milk, FCM, silage dry matter consumption, and body weight changes are presented. Of these measures, only the differences in silage consumption were significant, statistically. Cows fed the silages with the normal level of citrolas consumed the largest amount. This group was followed by those fed from the silo with the high level of additive. Those fed the control silage and that with the very high level of citrolas consumed about the same, and both were highly significantly lower than the two other groups. While the differences

TABLE 4

Milk production, F.C.M., silage consumption, and weight changes of cows fed the various silages

	Control	Normal-level citrolas	High-level citrolas	Very high-level citrolas
Milk *(lb/cow/day)*[a]				
3rd wk.	24.8	27.0	29.2	26.6
5th wk.	25.1	27.1	27.8	24.9
7th wk.	23.9	24.3	25.8	23.5
Average	24.6	26.1	27.6	25.0
FCM *(lb/day)(adjusted)*[a]	22.1	25.8	25.1	23.4
FCM *(7-wk. persistency—%)*	68.3	75.8	73.7	71.3
Silage dry matter intake *(lb/cow/day)*	18.6	22.1	21.0	19.0
Weight gains *(lb/cow/day)*	−0.6	−1.0	−1.1	−1.0

[a] Adjusted for slight differences in preliminary production by covariance procedure (2).

in milk and FCM production were not significant statistically, it is interesting that they tended to parallel the silage dry matter consumption.

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SUDAN GRASS SILAGE AT TWO STAGES OF MATURITY VERSUS RYE GRASS AND CRIMSON CLOVER WITH TWO FILLING PROCEDURES [1]

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SUMMARY

Tift Sudan grass silages, harvested at the preboot and the boot stages of maturity, were compared with rye grass and crimson clover silages which had been ensiled by a fast-filling procedure with packing and a slow-filling procedure without packing. Filling a silo slowly increased fermentation losses as measured by chemical changes and a balance study. Cows fed silage from the slowly filled silo consumed less dry matter, produced less milk, and gained less weight. When Tift Sudan grass was harvested at the preboot stage, milk production was higher, yields per acre lower, and seepage losses greater than when it was cut eight days later in the boot stage. Milk production and weight gains were higher for cows fed rye grass and crimson clover than for those fed Sudan grass silages. Fermentation losses were higher and seepage losses lower for the Sudan grass silages.

While both Tift Sudan grass and temporary winter-spring forages are important silage crops in the Southeast, information is largely lacking concerning their comparative values when used for this purpose. Tift Sudan grass has been shown to yield several times as much per acre when harvested as when grazed (6), but the effects of stage of maturity on its value for silage have not been adequately studied. Mixtures of rye grass and crimson clover either alone or with small grains are among the most-used combinations of temporary winter-spring forage. It has been shown previously that a mixture of the rye grass and crimson clover, alone, results in better-quality silage and greater economic returns when fed to lactating cows than does a combination of rye, rye grass, and crimson clover (7). There is a common belief that the quality of silage made from immature early-spring forages is improved by procedures designed to elevate the temperature during silage formation (2, 4, 5). However, there is a paucity of published research on the effectiveness of such practices.

In studies herein reported, silages made from Tift Sudan grass at preboot and boot stages of maturity were compared with rye grass and crimson clover silages ensiled by fast- and slow-filling procedures. The effect of these variables on temperature, and chemical changes during the ensiling process, nutrient losses, and feeding value for lactating dairy cows were studied.

EXPERIMENTAL PROCEDURE

Two 8- by 24-ft. upright silos were filled with a mixture consisting, on a dry matter basis, of Italian rye grass, 58%; crimson clover, 41%; and other, 1%. The rye grass was in the preboot stage and the crimson clover was approach-

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ing full bloom. One of the silos was filled April 29, between 11 A.M. and 5 P.M., with packing. The other was filled slowly, without packing, as follows: April 28, 29, 30, and May 5. Some of the material was left on wagons overnight and blown in the silo on the following day. Tift Sudan grass was put into two silos by the fast-filling procedure on July 22 and July 30, in the preboot and the boot stages of maturity, respectively. The yield per acre for rye grass and crimson clover was determined by weighing the material removed from a measured area. The yield of Sudan grass was determined by clipping ten randomly chosen plots of 1 sq. yd. each. Immediately following filling, each silo was covered with an 8-mm. polyvinyl cover and about 6 in. of sawdust was placed on top. The temperature measurements were made with copper-constantan thermocouples and a potentiometer. To prevent breaking, the thermocouple wires were put in the bottom door and placed at random about 2 to 4 ft. from the walls as the silos were filled.

Fifteen Holstein and eight Jersey cows were used to compare the four silages in a continuous type experiment with preliminary and post-experimental standardization periods. A 24th cow did not complete the study because of an injury unrelated to the treatment. The experimental silages were fed ad libitum as the sole roughage for 3 hr. twice daily for 6 wk. Immediately before and after the treatment period, grass silage was fed as the only roughage in 2-wk. standardization periods. During each of the three periods, milk production, fat test, and feed consumption were measured. Milk-O-Meters [2] were used to determine milk weights and obtain samples. For several weeks prior to the experiment the cows had been treated uniformly. The Morrison standard for the usual rate of feeding good hay (8) was used to determine the amount of concentrates to feed, based on FCM production during the period immediately preceding the standardization period. This level, which averaged 9.3 lb. per cow per day, was fed throughout the study. The concentrate mixture had the following chemical analyses: crude protein, 17.2%; crude fiber, 6.7%; ether extract, 2.5%; and ash, 6.2%.

Near the end of the treatment period the chromic oxide procedure was used to determine digestibility coefficients for the silages. Ten grams of chromic oxide was given to each Holstein cow at 8 A.M. during an eight-day preliminary and a six-day fecal collection period. The samples were analyzed for chromic oxide by the fusion method of Brisson (3). Body weights were taken after the cows had had a week to become adjusted to the experimental silages and at the end of the treatment period. This procedure has been shown to give more reliable results than taking the initial weights before the cows are fed the experimental silages (7). Other details of the chemical analyses, filling, sampling, and weighing procedures were as described previously (7) and were in conformity with recommended methods (1).

RESULTS AND DISCUSSION

The chemical composition of the various forages and silages is presented in Table 1. As would be expected, the Sudan grass harvested at the preboot stage

[2] Manufactured by Technical Industries, Fort Lauderdale, Florida.

TABLE 1

Chemical composition of the forages, silages, and seepage

	Rye grass–crimson clover		Tift Sudan	
	Fast-fill	Slow-fill	Preboot [a]	Boot [b]
Dry matter *(%)*				
Forage	12.2	13.1	12.8	16.4
Silage	18.7	18.8	14.9	17.0
Seepage	4.3	4.1	3.5	4.2
Crude protein *(% of d.m.[c])*				
Forage	16.1	14.9	12.8	9.5
Silage	16.4	16.7	15.0	11.2
Seepage *(% of seepage d.m.)*	41.5	39.6	44.7	25.0
Ether extract *(% of d.m.)*				
Forage	6.4	5.1	4.2	3.1
Silage	6.2	5.4	2.7	2.9
Crude fiber *(% of d.m.)*				
Forage	27.3	28.1	35.9	40.8
Silage	38.0	40.7	40.8	42.3
N.F.E. *(% of d.m.)*				
Forage	42.0	44.0	37.1	40.1
Silage	32.5	30.0	33.7	37.2
Ash *(% of d.m.)*				
Forage	8.2	7.9	10.0	6.5
Silage	6.9	7.2	7.8	6.4
Ammoniacal N *(% of d.m.)*				
(as NH_3)	0.7	1.0	1.1	0.5
pH of silage	4.6	4.8	5.1	4.8

[a] Preboot—Stage of maturity immediately preceding the boot stage.
[b] Boot—Seed heads are formed but are still enclosed in the sheath and thus are not visible.
[c] d.m. = Dry matter.

contained less dry matter and crude fiber but more protein, ash, and ether extract than the Sudan grass harvested in the boot stage. During the ensiling process there was a greater relative increase in the crude fiber content of the preboot Sudan grass. This silage also had a higher pH and contained more ammoniacal nitrogen, which indicated a less desirable fermentation than that made from Sudan grass harvested in the boot stage.

The rye grass and crimson clover forage was comparable to the preboot Sudan grass in dry matter content, but contained more protein and N.F.E. but less crude fiber and ash. During the ensiling process there was a greater increase in dry matter and crude fiber content in the rye grass and crimson clover. However, the reduction in N.F.E. during ensiling was much greater in the rye grass and clover mixture, whereas the protein did not change appreciably.

The average compositions of the fast- and slowly filled rye grass and crimson clover forages were similar. During the ensiling process there was a greater increase in the crude fiber and crude protein and more reduction in N.F.E. in the slowly filled silo. These changes, along with the slightly higher pH and ammoniacal nitrogen content, indicate that the slow-filling procedure resulted in more extensive fermentation and a lower-quality silage.

The nutrient losses during the ensiling process as measured by the balance study are presented in Table 2. Since there was no spoilage, the losses were

TABLE 2

Ensiling losses as measured by input-output data and dry matter yields

	Rye grass–crimson clover		Tift Sudan	
	Fast-fill	Slow-fill	Preboot	Boot
Ensiling losses				
Total weight *(% of forage wt.)*				
Seepage	40.4	38.3	32.5	9.1
Invisible	8.6	11.2	4.5	10.4
Total	49.0	49.5	37.0	19.5
Dry matter *(% of forage d.m.)*				
Seepage	14.5	12.5	8.0	2.2
Invisible	7.6	14.9	18.5	14.1
Total	22.1	27.4	26.5	16.3
Dry matter yields *(tons/acre)*				
Forage	1.53	1.53	1.44	1.98
Silage	1.19	1.11	1.06	1.66

limited to seepage, and the invisible or fermentation portion. The total dry matter loss from the preboot Sudan grass was much higher than from the boot-stage material. This difference was composed of a much larger seepage loss and a moderately higher fermentation loss. The total dry matter loss and the invisible portion were much greater from the slowly filled than from the rapidly filled silo, indicating that the slow rate of filling allowed much more fermentation.

Since the Sudan grass silages were made by the fast-filling procedure, it is perhaps most meaningful to compare these silages with the rye grass and crimson clover silage made by the rapid-filling technique. There was little difference in the moisture content between the preboot Sudan grass forage and that of the rye grass and crimson clover. Thus, the larger seepage losses from the rye grass and crimson clover probably reflect a true difference in the characteristics of the forages. Invisible losses were much larger in the Sudan silages. The total losses on a fresh basis were influenced predominantly by the amount of seepage.

Very little difference was detected in the temperatures which developed in the slowly filled and the rapidly filled silos. Maximum average temperatures for these two silages were 90 and 88° F., respectively, both of which are presumably well within a desirable range (5). The maximum average temperature which developed in the preboot and boot Sudan grass silages were 97 and 100° F., respectively. These somewhat higher temperatures in the Sudan grass silages, as compared to those made from the rye grass and crimson clover, may have been influenced by the warmer weather at that time of year. Several days after ensiling the forages, temperatures in all the silos began to decline slowly, reaching a minimum of approximately 60° F. in December.

Sudan grass harvested in the boot stage produced 37% more dry matter per acre than the preboot Sudan grass (Table 2). The greater production, together with the lower ensiling loss, resulted in an advantage of 57% in yields of silage dry matter per acre from the Sudan grass harvested in the boot stage.

The silage dry matter consumption data are presented in Figure 1. When the cows were switched from the standardization silage to the treatment silages

there was an immediate drop in silage consumption by all groups. This reduction in consumption was greatest for cows fed the boot-stage Sudan grass silage, second largest for the preboot Sudan grass, and about the same for the other two. Whereas, the other three groups soon reached a fairly stable level of silage consumption, those fed from the slowly filled silo decreased their consumption in a linear manner for the entire treatment period.

When the cows were again fed a standardization silage at the end of the treatment period, consumption was approximately equal for all groups. Thus, it appears that during the treatment period the slowly filled silage either was changing or the cows were continuing to adjust to it. Since the forage put in the bottom was a few days less mature than that at the top, consumption normally would not be expected to decline. Thus, it is reasonable to attribute the change in consumption to the slow-filling procedure. Leaching of the material in the bottom of the silo after it had already had a few days to ferment may have been a factor.

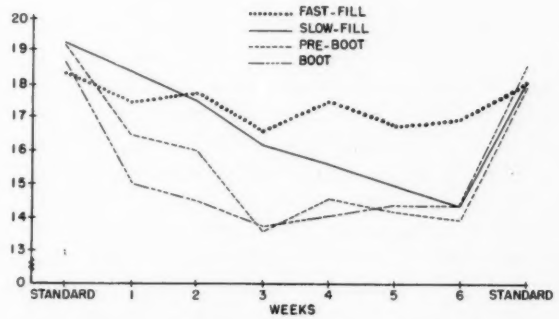

FIG. 1. Silage dry matter consumption (pounds per cow per day).

Cows fed the Sudan grass silage had a significantly lower persistency of milk production than those fed the rye grass and crimson clover material. Those given the boot-stage Sudan silage had a slightly lower persistency of production than those fed the preboot Sudan. Persistency of milk production of cows fed from the slowly filled silo was lower than that of those fed from the rapidly filled one. However, the decline in milk production did not follow the pattern of silage consumption, as the full differences in milk production, among cows fed the various experimental silages, were exhibited the second week the silages were fed. When the cows were switched from the experimental silages to the standardization silage, the increase in milk yields tended to be inversely proportional to the decline during the treatment period. Thus, the adverse effect of the slow-filling procedure on milk production was further verified. The weight gains of cows fed rye grass and crimson clover silage from the rapidly filled silo were significantly higher than any of the others (Table 3). Average weights of the various groups ranged from 957 to 989 lb. The differences in digestibility were not significant statistically ($P = 0.05$).

TABLE 3

Milk production, fat tests, FCM, persistency of milk production, weight gains, and digestibilities

	Rye grass–crimson clover		Tift Sudan	
	Fast-fill	Slow-fill	Preboot	Boot
Milk [a] *(lb/day)*	26.6	24.8	24.3	22.8
Fat test *(%)* [a]	4.33	4.05	4.29	4.30
FCM [a] *(lb/day)*	27.8	24.9	25.3	23.8
Persistency of milk *(%)* [b]	96.5	92.8	90.1	87.4
Change in milk to post-experiment period [c] *(%)*	102.0	111.0	105.5	110.0
Weight gains *(lb/day)*	1.1	0.1	−0.3	0.2
Digestibility *(dry matter)*	61.5	57.7	56.5	53.9

[a] Adjusted for slight differences in preliminary production.
[b] Persistency for 6 wk. adjusted to a 4-wk. basis.
[c] Milk production during second week of post-experimental standardization period divided by last week of treatment period times 100.

When all the measures are considered, it appears that the preboot Sudan grass silage was somewhat more nutritious than the boot-stage Sudan grass. However, the greatly increased yield and lower ensiling losses suggest that the later stage of harvest may be a more practical procedure on many farms.

As other factors may have had an important influence, only limited importance should be attached to the absolute differences in yields per acre between the Sudan grass and the rye grass and crimson clover silages. The data indicate that the slow-filling procedure was very detrimental from the standpoint of ensiling losses and animal performance. Since the slow-filling procedure is fairly comparable to that practiced on many farms, it is apparent that more studies are needed on the effect of rate of filling silos on silage fermentation, quality, and nutrient losses. Rate of filling may well be one of the important influences causing low-silage consumption on many farms. It also may be an important factor in explaining why considerable silage is of poor quality for no apparent reason.

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DIGESTIBILITY OF CHOPPED OAT SILAGE PRESERVED WITH ZINC BACITRACIN FED TO CATTLE AND SHEEP [1]

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SUMMARY

Chopped oat silage preserved with Silotracin (zinc bacitracin) was compared to a control oat silage without a preservative in a conventional total collection type digestion trial using both cattle and sheep. The percentage dry matter, protein, nitrogen-free extract, and ash content were higher in the Silotracin-treated silage than in the control silage, whereas the percentage of ether extract, crude fiber, and calories of gross energy content was lower. The Silotracin-treated oat silage contained 12% more crude protein and 11% less crude fiber than the untreated silage. Protein digestibility was significantly greater ($P < 0.01$) for the Silotracin-treated oat silage than for the control. Although there were no other statistically significant treatment differences in digestibility, there were increases in all digestion coefficients for the Silotracin-treated oat silage.

Several antibiotics have been used as preservatives for ensiling forages. A number of these antibiotics (aureomycin, bacitracin, penicillin, neomycin, streptomycin, terramycin, and various mixtures of these) were used at the levels of 2, 10, and 50 p.p.m. as preservatives for chopped alfalfa samples ensiled in quart jars or plastic bags (5). The silages treated with bacitracin, aureomycin, and streptomycin were generally excellent and mild in odor, whereas those with penicillin and neomycin were offensive in odor. Zinc bacitracin (4 to 40 p.p.m.) on 2 lb. of freshly chopped bromegrass-alfalfa forage ensiled in plastic bags produced highly satisfactory silage of good color, odor, and pH at the end of 90 days of storage (1). Oat forage ensiled in 1½-ton upright concrete experimental silos using 5 g/ton bacitracin as the preservative produced silage of good quality (11, 13). The palatability studies showed a preference for the antibiotic-preserved silage.

The objective of the study reported herein was to compare the digestibility of nutrients in chopped oat silage with no preservative (control) to a silage containing Silotracin (zinc bacitracin) as a preservative.

EXPERIMENTAL PROCEDURE

Oat silage was made from Floriland oats grown on a fine sandy soil and cut at the prebloom stage of maturity. It was ensiled in upright-type polyvinyl [3]

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[3] Polyvinyl furnished by Union Carbide Company, New York, New York.

experimental silos 4 ft. high and 4 ft. 8 in. in diameter. These silos were supported with wire and sealed off at the top. One silage with no preservative (control) was compared with another silage treated with 5 g. of zinc bacitracin per ton of fresh chopped oat forage. The oats remained ensiled for a period of 53 days.

One digestion trial was carried out with four crossbred Native-Rambouillet 1-yr.-old wethers of similar weight, which were fed in expanded metal collection racks. In a second trial, four nonpregnant 4-yr.-old cows of similar weight were fed in stanchion-type stalls and feces were collected as described by Gorski *et al.* (6).

The digestion trials were of a conventional total collection continuous type and each period consisted of a seven-day adjustment period, a ten-day preliminary period, and a seven-day collection period. A 2 × 2 Latin-square design was used with each species of animals and each was replicated. The animals were fed oat silage (ad libitum) during a seven-day adjustment period, to determine the quantity of silage they would consume. During the preliminary and collection periods the animals were fed oat silage at a constant rate. Trace-mineralized salt and defluorinated phosphate were fed to meet the requirements of the animals and water was available at all times. Feed and feces were weighed twice daily and aliquot samples were dried in a forced air oven at 65° C. for 72 hr. The samples were allowed to equilibrate to air-dry conditions at room temperature for three days and were then weighed for dry matter, ground, and samples weighed and composited for chemical analyses.

Proximate chemical analyses were made as outlined by the A.O.A.C. (2). Nitrogen-free extract was determined empirically by difference and gross energy was measured with an adiabatic oxygen bomb calorimeter. Analysis of variance was conducted as described by Snedecor (15).

RESULTS AND DISCUSSION

The average chemical composition and gross energy content of the chopped oat silages are summarized in Table 1. The percentage of dry matter, protein,

TABLE 1

Average chemical composition and gross energy content of chopped oat silage in plastic silos (moisture-free basis)

Preservative treatment		Dry matter in silage	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Gross energy
		(%)						(Kcal/g)
Silotracin 5 g/ton	I [a]	13.4	17.5	6.1	30.3	36.1	10.1	4.3
	II [b]	13.7	17.0	7.1	30.3	35.6	10.0	4.4
	Av.	13.5	17.2	6.6	30.3	35.9	10.0	4.4
None (Control)	I [a]	11.6	13.8	6.6	35.3	37.0	7.4	4.3
	II [b]	12.7	16.3	7.4	32.8	33.9	9.6	4.7
	Av.	12.2	15.1	7.0	34.0	35.4	8.5	4.5

[a] Represents a composite sample of the top half of the plastic silos.
[b] Represents a composite sample of the bottom half of the plastic silos.

nitrogen-free extract, and ash was higher in the Silotracin-treated silage than in the control silage. However, the percentage of ether extract, crude fiber, and calories of gross energy was lower in the Silotracin-treated silage than in the control silage. Similar results have been reported by Andrews and Stob (1), who used 4 g. of zinc bacitracin plus 40 lb. of molasses per ton of oat silage.

The Silotracin-treated oat silage contained 12% more crude protein than the untreated silage. This increase may be due to the prevention of loss of nitrogen as ammonia due to putrefaction of proteins and amino acids, as indicated by less ammonium odor in the Silotracin-treated silo.

Since a total weight of the silage was not taken before or after ensiling, it would be impossible to pinpoint the reason for the 11% decrease in crude fiber content of the Silotracin-treated silage as compared to the control.

There was an interesting relationship between the condition of the silage in the top and bottom half of the silos and the chemical composition (Table 1). The silage in the bottom half of the untreated silo was of better quality than that in the top half. This was not the case in the Silotracin-treated silo, in which the chemical composition was relatively constant throughout. This may indicate that in the top half of this silo the fermentation process was enhanced by the Silotracin preservative. From these data, it would appear then that the top portion of the silo would be the logical place to concentrate the preservative.

While both the treated and untreated silages were lacking in palatability, the Silotracin-treated silage was more palatable than the control silage, as measured by animal acceptance and consumption. It took the animals a few days to become accustomed to eating the silage; however, the sheep consumed the silage more readily than did the cows. The Silotracin-treated silage had a more desirable color and odor than the control silage. The pH of composite samples from the bottom half of each silo was 5.6 for the control and 5.3 for the Silotracin treatment. Other workers have reported that silage made with Silotracin as a preservative was of good color and odor and that the palatability was enhanced (1, 5, 11). However, Becker *et al.* (3), feeding oat forage from the same source as reported herein, found that large dairy heifers consumed less Silotracin-treated oat silage than a control oat silage. The average amounts of oat silage consumed per day per heifer were: no preservative 98 lb., Silotracin 87 lb., and ground snapped corn 81 lb.

The results of the digestion trials are given in Table 2. There was a highly significant increase ($P < 0.01$) in protein digestibility of the Silotracin-treated silage over the control silage. Although there were no other statistically significant treatment differences in nutrient digestibility, there were trends indicating advantages for the Silotracin-treated silage.

One explanation for the increased digestibility of nutrients may be a more favorable environment in the rumen for cellulose breakdown and volatile fatty acid production when the Silotracin-treated silage was fed. This is apparent since Rusoff (11) reported that lactic and propionic acid content of oat silage dry matter was greater in a Silotracin-treated silage than in a control oat silage.

Ramsey *et al.* (10) reported no significant difference in the digestibility of

nutrients in oat silage preserved with Silotracin from that of a control oat silage without a preservative. Work with other forages (4, 9, 12, 13, 16) has not shown any significant advantage in digestibility or fat-corrected milk (FCM) production when Silotracin was used as a preservative.

TABLE 2

Average apparent digestion coefficients, digestible energy, and total digestible nutrients for chopped oat silage by cattle and by sheep

Preservative treatment		Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Energy	TDN	Digestible energy
		(%)							(Kcal/g)
Silotracin	Wethers [a]	58.0	63.1	53.1	77.7	60.2	62.1	63.6	2.7
5 g/ton	Cows [a]	55.9	61.0	51.3	77.2	52.7	56.0	60.5	2.5
	Av.[b]	57.0	62.1**	52.2	77.5	56.5	59.1	62.1	2.6
None	Wethers [a]	54.6	54.9	52.8	74.4	55.7	58.6	61.7	2.5
(Control)	Cows [a]	51.8	50.2	42.7	76.7	53.7	54.3	59.6	2.6
	Av.[b]	53.2	52.6	47.8	75.6	54.7	56.5	60.7	2.6

[a] Each digestion coefficient is the mean of four observations on either cows or wethers.
[b] Each digestion coefficient represents the mean of eight observations on both cows and wethers.
** ($P < 0.01$).

Data present in Table 2 indicated that the crude fiber fraction was more digestible than the nitrogen-free extract (NFE). Similar findings are reported in the composition tables published by Morrison (7) and Schneider (14). The indigestible lignin fraction of feed is usually in the crude fiber fraction, whereas the readily digestible carbohydrates are usually in the NFE fraction; however, Moxon and Bentley (8) have reported that lignin is often found in larger amounts in the NFE fraction than in the crude fiber fraction. Thus, the conventional Weende method (A.O.A.C.) of forage analysis often may provide misleading information relative to the nutritive value of the crude fiber content of some forages.

These studies indicate that Silotracin can be used to advantage as a preservative of oat silage cut at a very early stage of maturity.

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TECHNICAL NOTES

ESTABLISHING THE PRESENCE OF DISTEARYL TRIGLYCERIDES IN A HIGH-MELTING FRACTION OF MILK FAT USING SILICIC ACID CHROMATOGRAPHY [1, 2]

Milk fat contains an almost infinite number of different triglycerides only a few of which have been separated in sufficient concentration to make positive identification possible. Attempts to establish the presence of specific triglycerides involve extensively fractionation of the fat, using a variety of methods. During the course of an investigation of a high-melting glyceride fraction (HMGF), a silicic acid chromatography method was developed that was particularly effective in concentrating the stearate-rich triglycerides. The method, herein described, was used to establish the presence of disteraryl triglycerides in milk fat.

HMGF was obtained by holding a solution of 400 g. of butteroil and two liters of acetone at −2° C. for 12 hr. The precipitate was washed with cold solvent, redissolved in 800 ml. of acetone, and held overnight at room temperature. The crystalline material recovered by filtration was washed with acetone. Evaporation of the occluded solvent yielded 20 g. of HMGF.

HMGF was fractionated, using a modification of the Hirsch and Ahrens (2) silicic acid procedure developed for the separation of complex lipids. Their method suggested that some partitioning of the triglycerides could be obtained, particularly if the triglyceride mixture was relatively simple. The Hirsch and Ahrens instructions for silicic acid preparation were followed, except that the adsorbent was not milled and the settling periods for the silicic acid–solvent suspensions were reduced from 30 to 15 min. These modifications decreased solvent flow rate. Column temperature was not closely controlled.

The column, consisting of 18 g. of silicic acid packed in a 15- by 750-mm. glass tube, was washed successively with 20 ml. of ethyl ether, 40 ml. of acetone–ethyl ether (1:1, V:V), 20 ml. of ethyl ether, and 300 ml. of petroleum ether. Solvent flow rate was approximately 60 ml. per hour. The column was charged with 0.3 g. of HMGF dissolved in 4 ml. of petroleum ether, and elution solvents were 1, 2, 3, and 4% solutions of ethyl ether in petroleum ether. Any lipid material remaining on the column after this treatment was eluted with 100% ethyl ether. Fraction volumes varied, depending on the objectives of the experiment.

Triglyceride fractions were converted to methyl esters via alkaline saponification and H_2SO_4 catalyzed esterification with methanol. A Barber-Coleman Model 10 instrument was used for gas chromatographic analysis. Operating procedure was according to Patton *et al.* (4).

Table 1 lists data collected when 0.3 g. of HMGF was subjected to silicic acid chromatography, using between 100 and 200 ml. of each solvent combination. Initial experiments revealed that the fastest-moving lipid fractions were richest in stearate triglycerides. Since these triglycerides were of primary importance in this study, fractions were collected in smaller volumes (40 ml.) when such material was being eluted from the column. The slower-moving components of the HMGF, being of secondary interest, were collected in larger fractions.

TABLE 1

Silicic acid chromatography of HMGF

Fraction	A	B	C	D	E	F	G	H	I	J
% Ethyl ether in eluting solvent	1	2	2	2	2	2	3	3	4	100
Milliliters in fraction	250	40	40	40	40	40	50	80	180	100
Milligrams in fraction	0	8	35	32	35	30	46	44	25	6
Per cent of HMGF weight	0	3	13	12	13	12	18	17	10	2

Table 1 indicates that silicic acid chromatography did not partition the HMGF sufficiently to give any major elution peaks. Other trials have been conducted in which 10-ml. fractions were collected. These trials demonstrated

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[2] Supported in part by U. S. Public Health Service (H3632).

TABLE 2

Fatty acid distribution in triglyceride fractions obtained by silicic acid chromatography of the HMGF [a]

Fraction	Original HMGF	B	C	D	E	F	G	H	I	J
Acid chain length										
C_{10}	0.6	0.4	.....	.....	.....	.....	0.2	1.3	4.7	.....
C_{12}	1.8	0.5	0.1	0.3	0.4	0.7	1.6	3.8	7.0	.....
C_{14}	10.5	4.5	4.4	8.6	7.9	10.9	14.8	15.9	19.5	.....
C_{16}	43.3	32.4	35.8	42.5	43.2	47.6	48.3	49.9	45.8	.....
C_{18}	29.2	50.0	50.2	38.1	35.2	27.9	23.5	16.0	13.3	.....
C_{20}	Trace	3.5	2.4	1.0	.....	.....	.....	.....	.....	.....
C_{18} unsaturated	7.6	6.9	6.0	7.1	7.0	8.1	7.4	9.0	7.7	.....
Misc. peaks	7.0	5.2	3.6	2.9	5.9	4.2	3.6	3.4	1.5	.....

[a] Per cent by weight.

a better separation of triglycerides than that indicated in Table 2. Several broad, major peak areas were noted. However, even when small fractions were collected the over-all elution pattern was gradual, indicating that the isolation of a specific triglyceride could not be realized. Such a separation could hardly be expected, since the HMGF contains a multiplicity of different triglycerides with very similar solubility and adsorption characteristics.

Table 2 lists the fatty acid distribution in each triglyceride fraction obtained by silicic acid chromatography. The gas chromatographic methyl ester analysis revealed that the silicic acid column was effecting a gradual separation of the triglycerides in the high-melting fraction. The high stearate glycerides were eluted first. The stearate content of the early fractions was about 50% and each succeeding fraction contained progressively less of the C_{18} saturated acid. The stearate level of the slowest-moving fraction was only 13%. Accompanying this marked decrease in stearate concentration was a gradual increase in the lower molecular weight C_{16} and C_{14} saturated acids. The slowest-moving fractions even contained considerable quantities of C_{10} and C_{12} acids. It is evident that the column fractionated the triglycerides according to molecular weight, with the highest molecular weight material being eluted first.

Since several fractions contained more than 33% stearic acid, the presence in milk fat of traces of distearyl triglycerides, particularly palmitadistearin, was established beyond any reasonable doubt. Of course, Table 2 also indicates that HMGF contains dipalmityl triglycerides. The presence of such glycerides had been well established previously (1, 4, 5).

Rechromatographing of the stearate-rich portion of the HMGF did not yield any lipid material with more than 50% stearic acid. Thus, it can be concluded that milk fat does not contain any tristearin. If tristearin were a component it would concentrate in the HMGF, and it should be possible to obtain a fraction from the silicic acid column with greater than the 67% stearic acid concentration required for mathematical proof. This reasoning is based on the fact that tristearin has a molecular weight that is greater than practically any conceivable triglyceride of the HMGF. This difference would give tristearin a chromatographic behavior sufficiently unique to make possible the collection of a very rich stearate fraction. However, several attempts to separate a fraction with more than 50% stearic acid failed.

Admittedly, silicic acid will give at best only a crude separation of the triglycerides in any milk fat fraction. However, it does have considerable versatility and by slight adjustments of solvent combinations other fat fractions can be separated. It has been applied to fractions rich in low molecular weight glycerides with considerable success.

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CAUSE OF PREFREEZING IN THE FREEZING POINT DETERMINATION OF MILK

During an investigation on the freezing point of milk in Alberta, some samples of milk were found to show prefreezing, which makes the accurate determinations of the freezing point impossible. A similar difficulty has recently been described by Sargent *et al.* (1), who attributed it to the action of bacteria. In the present investigation, a Fiske Model I cryoscope was used. The freezing-point determination involves supercooling the milk and subsequent freezing by the violent stirring action of a metal rod. The temperature then rises to the freezing point and remains constant. The abnormal samples could not be supercooled and the milk froze before any supercooling was achieved. As the initiation of freezing requires the formation of centers of crystallization, it was surmised that the abnormal samples were contaminated by solid particles that would act as crystallization centers. The prefreezing phenomenon occurred only in the milk from a small number of producers, occasionally in the milk from a tank truck, but never in retail milk sent out from the local dairy plants.

An inspection on some of the farms involved revealed nothing of an unusual nature in cleaning procedures and use of materials. Attention was finally drawn to the well-water used on these farms. It was found to be responsible for the prefreezing of the milk. A sample of well-water obtained from a farm where prefreezing of the milk was encountered could be added to normal milk at the level of ½% to cause prefreezing. Filtration of the abnormal milks through fine glass and paper filters did not cause a change in the prefreezing. Ultrafiltration of abnormal milks resulted in ultrafiltrates that froze normally. However, ultrafiltration can not be used to measure freezing points of abnormal milks, because the freezing points of milk and its ultrafiltrate are not identical. This phenomenon will be the subject of a further publication. Microscopic examination of the abnormal milk and the well-water revealed no discernible solid particles. After evaporation to dryness, the well-water could be shown to contain solid mineral particles of irregular shape and with sharp edges. The content of dry matter in the well-waters ranged from 800-1,000 p.p.m.

The sediment in these waters apparently settles easily when the equipment such as bulk tank is rinsed and, even when very little water is left in the tank there is sufficient solid material to cause the milk to show prefreezing. This also explains why the abnormality occurs only in certain shipments and never in retail samples. The dilution of the sediment in the dairy plant assures that the level in retail milk is insufficient to cause prefreezing.

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ELECTRONIC HUMIDITY ELEMENT FOR MEASUREMENT OF RELATIVE VAPOR PRESSURES [1, 2]

A method that has been useful in evaluating the water sorption of milk proteins is reported herein. It is based on the measurement of relative vapor pressures by a lithium chloride–polyvinyl alcohol, electronic, humidity-sensing element.

The operating setup of the equipment used in the determination of relative vapor pressure is shown in Figure 1. The microammeter on the left is connected to the humidity-sensing unit, attached inside the lid of the sample jar

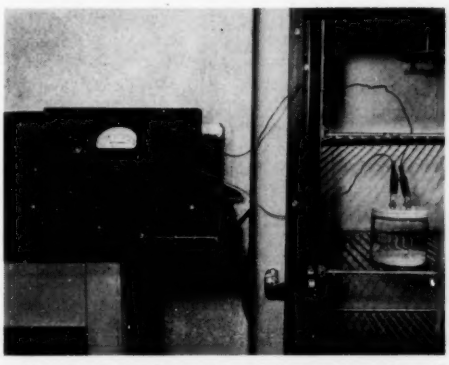

Fig. 1. Operating setup of the equipment used in the measurement of relative vapor pressures.

[1] Scientific Journal Series Paper No. 4569, Minnesota Agricultural Experiment Station, St. Paul.

[2] Data presented are taken from a thesis by the senior author submitted to the Graduate School of the University of Minnesota, in partial fulfillment of the requirements for the Ph.D. degree, August, 1960.

shown in the incubator. A more detailed description of the individual components follows.

Relative humidity-sensing elements. The relative humidity-sensing elements were developed and are manufactured by the Minneapolis-Honeywell Regulator Company, 2753 Fourth Avenue South, Minneapolis, Minnesota, and are referred to as Q-229AY Electronic Humidity Elements. They are described in that company's Applied Research Project 4799 Report 1.

Elements ranging from 12 to 99% relative humidity were used. Each element covers a short range of relative humidity to from 10 to 18%.

The elements were specially calibrated by the manufacturer, by use of a Precision Atmosphere Producer, to yield an accuracy of ± 0.5% relative humidity. Since the relative humidity, as measured by these elements, is a function of the resistance to a flow of current across the electrodes, the resistance values measured throughout the range of relative humidity by a particular element result in a calibration curve for that element. The manufacturer provides calibration curves for each element. The calibration is described in Minneapolis-Honeywell Research Report Gr 2998-R1.

The elements were checked periodically for changes in calibration by using various saturated salt solutions (3).

The relative humidity of the air in each chamber at equilibrium, as measured by these elements, was used as a measure of the relative vapor pressure of the water associated with the sample. This was possible since the sample and sensing elements were isolated in a vapor-tight chamber at a constant temperature. This is the first reported use of the elements to measure relative vapor pressures.

Relative vapor pressure chambers. The relative vapor pressure chambers consisted of glass jars 8.5 cm. deep and 7.5 cm. in diameter. These jars were sealed by a metal screw cap fitted with an attached latex gasket. Preliminary investigation verified the vapor-tight seal of this chamber. The banana-plug electrical connectors were sealed in holes drilled through the cap and were isolated from the metal by rubber gaskets. An electronic humidity element was attached to the banana plugs inside the lid of the chamber. An inside view of the screw cap and the attached element is shown in Figure 2.

Microammeter. Resistances were measured by a three-range microammeter with a self-contained power supply. The microammeter was built by the Minneapolis-Honeywell Regulator Company. The manufacturer provided calibration curves of microamperes versus megohms resistance. The device was further checked in this laboratory by use of standard resistances.

Incubator. Temperature of the relative vapor pressure chamber with its enclosed sample and humidity element was controlled at 20 ± 0.1° C.

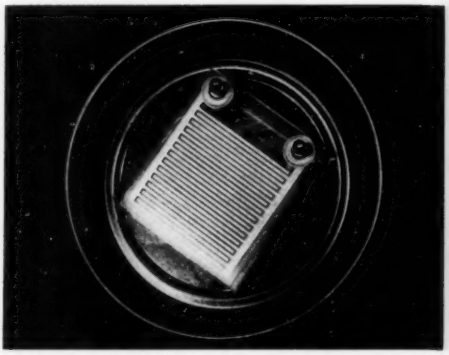

FIG. 2. An inside view of the lid of the R.V.P. chamber, showing the sensing element attached to the electrical connectors.

in a Ceneo forced-air incubator. The incubator was located in a refrigerated room, the temperature of which was controlled at about 2° C. less than the incubator setting.

EXPERIMENTAL PROCEDURE

Preparation of samples. Samples of various casein preparations were evaluated. These preparations were freeze-dried and subsequently rehydrated by exposing to steam for different periods of time, which resulted in samples having varying moisture contents and varying vapor pressures.

Relative vapor pressure measurements. The vapor pressure chambers with their enclosed samples (1 g. of casein preparation) were held in the incubator for ten days, with daily mixing accomplished by shaking the containers. At the end of this period of time, blank lids were quickly replaced with lids to which the elements were attached. After 24 hr., and for the next 48 to 72 hr., the samples were monitored and the readings recorded at 24-hr. intervals. When the readings became constant, the relative vapor pressures corresponding to those readings were recorded for the sample.

Moisture determination. Moisture was determined according to the method for cheese described in Official Methods of Analysis (1). Preparations were sampled for moisture analysis immediately after the completion of the relative vapor pressure measurements. All weighings were made with a Sartorius Selecta Rapid Model Balance.

RESULTS AND DISCUSSION

Plots of data obtained on casein and sodium caseinate through the use of the elements, along with data obtained through the use of an isotenoscope by Briggs (2), are presented in Figures 3 and 4. The excellent agreement between

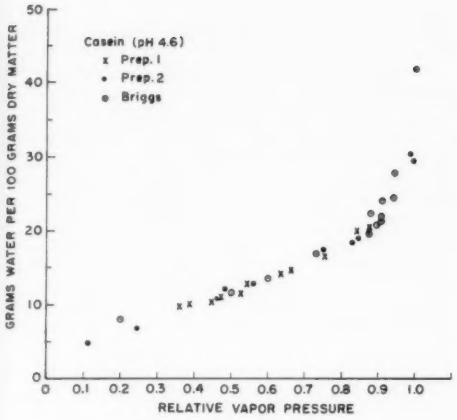

FIG. 3. Vapor pressure—moisture content curves for casein. A comparison of Brigg's data (2) and data obtained through the use of electronic humidity elements.

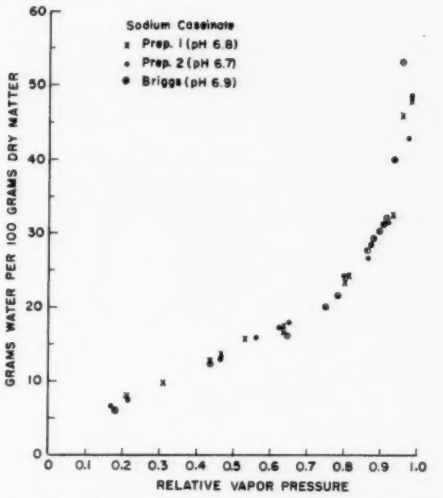

FIG. 4. Vapor pressure—moisture content curves for sodium caseinate. A comparison of Brigg's data (2) and data obtained through the use of electronic humidity elements.

the two methods is readily apparent.

Use of the humidity elements is comparatively simple and through the use of many elements a large number of equilibrated samples can be analyzed in a relatively short period of time.

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EFFECTS OF ENVIRONMENTAL TEMPERATURE AND HUMIDITY ON THE FATTY ACID COMPOSITION OF MILK FAT [1]

There is a great deal of general information concerning changes in the characteristics of milk fat secreted by the dairy cow under the influence of various environmental conditions. The Reichert-Meissl value has been reported to decline and the iodine number increase between 90 and 95° F. (6). Other investigations have indicated that some influence connected with a change in the environmental temperature affected the unsaturated acid content of milk fat (1, 2, 5, 8, 10).

Past evaluations of the effects of specific environmental factors on the fatty acid composition of milk fat have been obscured by lack of control of all conditions surrounding the production of milk. Normally, seasonal changes were accompanied by simultaneous changes in feed quality and stages of lactation. There was seldom any control of the environmental temperature and humidity during the studies cited. Furthermore, practically all investigations to date have been accomplished through the use of the so-called fat constants which are estimators of certain groups of fatty acids.

This experiment was designed to eliminate all variables (quality of feed, differences in stage of lactation, seasonal changes, and others) except temperature and humidity, and to employ a new gas chromatographic procedure for analysis of milk fat samples obtained at various temperature and humidity conditions.

EXPERIMENTAL METHODS

The gas chromatographic procedure used in this study has been described in detail by Gehrke *et al.* (3). Seven basic steps are involved

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 2241. Approved by director.

in the method: (a) Extraction of the lipid material from the milk sample, (b) saponification, (c) removal of alcohol, (d) extraction of free acids with ethyl ether, (e) formation of silver salts, (f) drying of the silver salts and esterification with methyl iodide, and (g) instrumental analysis.

It was necessary to maintain a positive nitrogen atmosphere throughout the entire procedure for the analysis of milk fat to prevent oxidative deterioration. The instrument used for analysis was the Perkin-Elmer 154C gas chromatograph, employing a 1-mm. column packed with 20% Apiezon L grease on Celite 545.

Butyric acid passed through the column too rapidly for the recorder to characterize accurately; therefore, it was not reported in this investigation. It was assumed that butyric acid would follow the same trend as the other short-chain fatty acids (C_6-C_{12}). Oleic acid ($C_{18}\Delta^9$) and stearic acid (C_{18}) were incompletely separated by the column and conditions used in this study. Chromatograms were made of the pure methyl esters of each acid separately and in combination. This provided a graphic separation of the two overlapping areas. By this procedure, the two areas identified as oleic and stearic acids were estimated. Chromatographic areas designated as oleic acid include small amounts of linoleic and linolenic acids.

The lactating Holstein cows used in this study were housed in the climatic laboratory at the University of Missouri. One chamber in the climatic laboratory was maintained at 65° F. and 50% relative humidity continuously. The other chamber was used as the test chamber and was maintained within a very narrow range of the temperature and humidity desired for each environmental treatment. Each cow served as its own control.

A switch-back design was employed. For example, animals were exposed for 2 wk. to the control 65° F., then exposed to a treatment for 2 wk., after which they were returned to the control conditions. Four animals in each stage of lactation (early, mid, and late) were used in this study. Milk samples were frozen soon after milking to reduce the possibility of lipase action on the fat, and stored at −5° C. until analyzed.

A number of milk samples were collected from individual cows showing varied degrees of thermal stress during the treatment conditions. These samples made up the bulk of this study. The degree of thermal stress was judged by increases in rectal temperature, decreases in feed consumption, and general symptoms of discomfort. Some samples from cows exhibiting very little, if any, heat stress were also analyzed.

RESULTS AND DISCUSSION

Declines in feed consumption and losses in body weight were characteristic of most cows at the high temperature–humidity levels (Johnson *et al.*, unpublished data). Figures 1 and 2

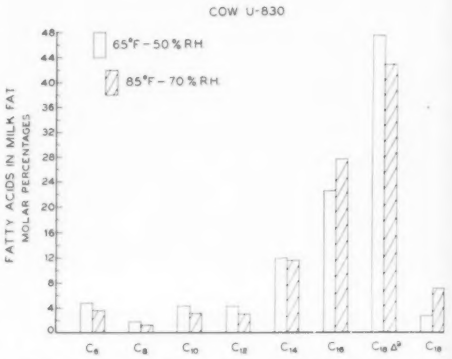

FIG. 1. Comparison of the relative proportions of fatty acids in the milk fat of a Holstein cow produced at 85° F.—70% R.H. and at the control conditions (65° F.—50% R.H.). (The comparison of the fatty acids produced by this cow under the environmental conditions described are characteristic of most of the results obtained in this investigation.)

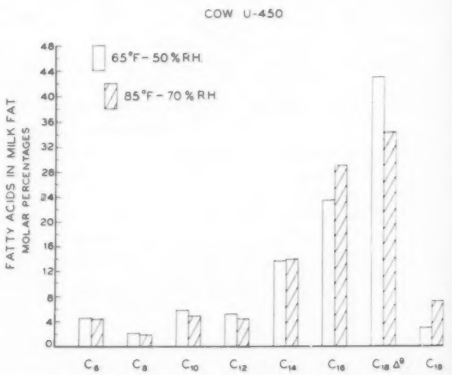

FIG. 2. Comparison of the relative proportions of the fatty acids in the milk fat of a Holstein cow produced at 85° F.—70% R.H. and at the control conditions (65° F.—50% R.H.). (The comparison of the fatty acids produced by this cow under the environmental conditions described are characteristic of most of the results obtained in this investigation.)

present graphic illustrations characteristic of most of the results obtained in this study. These figures constitute the molar percentage changes observed in the fatty acid composition of milk fat from Cows U-830 and U-450 at 85° F.—70% R.H., as compared to the control conditions (65° F.—50% R.H.). The production of the short-chain fatty acids (C_6-C_{12}) was depressed at the higher temperature and hu-

midity level. The differences between the short-chain fatty acids from all analyses in this investigation were combined and subjected to a statistical analysis ("t" test). The decrease in the short-chain fatty acids at the higher temperature and humidity conditions, when compared to 65° F.—50% R.H., were significant at the 1% level. Slightly less myristic acid (C_{14}) was produced by U-830, whereas U-450 produced slightly more at the treatment conditions. The combined statistical analysis for myristic acid revealed no significant difference between the treatment and control conditions. A considerable increase in palmitic acid (C_{16}) occurred at 85° F.—70% R.H. A similar increase in palmitic which was significant at the 1% level was observed at all treatment conditions, as compared to the control for every animal in this experiment.

A reciprocal relationship was observed between oleic acid ($C_{18}\Delta^9$) and stearic acid (C_{18}). Oleic acid decreased at higher temperatures whereas stearic acid increased. The combined statistical analysis showed the decline in oleic acid to be significant at the 5% level and the increase in stearic acid significant at the 1% level. Relative total amounts of the C_{18} acids (oleic and stearic) combined were essentially the same at 85° F.—70% R.H. and at 65° F.—50% R.H. The major difference seemed to be a shift in the degree of unsaturation at the higher temperature and humidity levels. This same reciprocal relation between the C_{18} acids was observed in all but two analyses. Hilditch (5) has reported that such a reciprocal relationship does exist in the milk fat as well as in the depot fats of cows.

A comparison was made between early and late lactation. This was done to study any influence of stage of lactation, or level of production, on the fatty acid composition of milk fat. The same general reactions were observed in these analyses as have been described for Cows U-830 and U-450. The main difference was that late-lactation cows produced slightly more oleic acid at the higher temperature and humidity level. Actually, the differences exhibited between the proportions of each fatty acid produced at early and late lactation were small. The early group produced slightly more palmitic and yielded slightly less oleic at the higher treatment conditions.

The statistical analysis, as indicated previously, showed significant effects of environmental conditions above 65° F. However, the molar percentages of fatty acids produced by the animals at 85° F.—70% R.H., 90° F.—25% R.H., and 95° F.—25% R.H. were similar and, thus, not significantly different.

Results obtained in this study indicate that there are differences in the fatty acid composition of milk fat from cows held continuously at various controlled environmental conditions. Differences were observed in the relative proportions of fatty acids in milk fat produced at higher temperature and humidity levels (85° F.—70% R.H., 90° F.—25% R.H., and 95° F.—25% R.H.), as compared to the proportions produced at environmental conditions considered to be within the optimum range for the production of milk (65° F.—50% R.H.). The results indicate that any combination of high environmental temperature and humidity which result in a rectal temperature of 103 to 105° F. may cause definite changes in the characteristics of the milk fat. After the rectal temperature has reached this approximate level, and the characteristics of milk fat have changed, it appears that any moderate increase in the environmental temperature and humidity may have little additional effect upon the composition of milk fat.

The observation that differences do exist in the fatty acid composition of cows' milk fat is in agreement with seasonal studies by Cox and McDowall (1), Frye *et al.* (2), Hansen and Shorland (4), Stadhouders and Mulder (8, 9), Hilditch (5), Reinart and Nesbitt (7), Wood and Haab (10), and a controlled environmental study by Regan and Richardson (6). Some differences observed in certain individual acids do not agree with the findings of these investigators.

The decrease in short-chain fatty acids (C_6-C_{12}) at environmental temperatures ranging from 85 to 95° F. with 25 to 70% R.H. is in agreement with a controlled environmental study by Regan and Richardson (6). These investigators reported a lower Reichert-Meissl value, which is a measure of the short-chain fatty acids, at temperatures between 90 and 95° F. Cox and McDowall (1) reported a lower Reichert-Meissl value for milk fat produced during the warmer months of the year.

The decrease in oleic acid at the higher environmental conditions is in contrast to many of the seasonal studies in which the iodine number has been used as an index to the degree of unsaturation in milk fat, Frye *et al.* (2), Stadhouders and Mulder (8), and Wood and Haab (10). It should be emphasized that iodine number measures the total degree of unsaturation in milk fat. Oleic acid is the chief unsaturated acid in milk, but the iodine number is also affected by the amounts of all other unsaturated acids present. More recent research, on the method used in this study, has revealed a number of smaller peaks which may be unsaturated counterparts of the chief fatty acids in milk. Further research is needed in this area to clarify the conflicting evidence between the proportions of oleic acid found in this study and iodine values reported in the literature.

It is interesting to note that the fatty acids with the lower melting points decreased at the higher temperature and humidity levels. These included the short-chain fatty acids (C_6-C_{12}), with melting points varying between −1.5° C. and 47° C., and oleic acid ($C_{18}\Delta^9$) with a melting point of 14° C. The two fatty acids with

the highest melting points, palmitic (63° C.) and stearic (69° C.), increased at the higher environmental conditions.

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BOUND ALDEHYDES IN BUTTEROIL

In connection with studies on off-flavor development in dry whole milk, substantial quantities of fatty aldehyde 2,4-dinitrophenylhydrazones were obtained by passing the extracted lipids (2) through an acid solution of 2,4-dinitrophenylhydrazine, according to the procedure of Schwartz *et al.* (8). Based on previously reported (6) threshold studies, and the flavors of the products investigated, it was concluded and later substantiated that the greater majority of these aldehydes were bound in butteroil.

At the time of this find, bound aldehydes in butteroil had not been reported, although van Duin (10) reported the liberation of aldehydes from the plasmalogen fraction of butter serum. However, during the later stages of this investigation, Schogt *et al.* (7) reported the isolation of a phosphorus-free lipid from butteroil which contained aldehydes bound to glycerol in an enol-ether linkage. This finding confirmed our belief as to the nature of the aldehyde precursor, based on the observation of Day and Lillard (1) that phosphorus was not detectable in butteroil. Schogt *et al.* (7) reported 40 mg. of bound aldehyde per kilogram of fat expressed as myristaldehyle, which compares favorably with our finding of 0.2 μM bound aldehyde per gram of butteroil.

Our preliminary results on the bound, saturated aldehydes as their 2,4-dinitrophenylhydrazones suggested a complex mixture which was not resolved by the available column chromatographic techniques. Therefore, gas chromatographic techniques were introduced.

The butteroil used in these studies was obtained by melting sweet cream butter at 100° F. and centrifuging in an International centrifuge. The butter was prepared by churning cream obtained from fresh milk. No more than two days time elapsed between milking and conversion to butteroil. The oil was analyzed immediately after preparation. Analysis of oil stored at 0° F. for 2 mo. yielded the same results as the fresh oil.

The method for isolating the aldehydes liberated from the nonphospholipid material was as follows: 800 ml. of a 25% solution of butteroil in carbonyl-free hexane[1] was dried with sodium sulphate, filtered, and passed through a chromatographic tube containing three layers of analytical grade Celite[2] (Johns-Manville Company) separated by glass wool. Layers One and Three contained 20 g. of Celite impregnated with 12 ml. of a saturated solution of sodium

[1] Hexane (Phillips, High Purity Grade) scrubbed through a 5-ft. column of concentrated H_2SO_4, followed by redistillation from KOH pellets (3). Analysis of the purified hexane indicated that it contained 0.65 μM of carbonyl per liter.

[2] The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

bisulphite, whereas Layer Two consisted of 20 g. of celite containing 20 ml. of a 50% solution of phosphoric acid. The column was packed so that a flow rate of less than 1 ml. per minute was attained. Following passage of the butteroil-hexane solution, the packings were washed free of fat with purified hexane. The lower bisulphite packing was removed from the column and the aldehydes freed by decomposing the bisulphite with 20% sodium carbonate. The aldehydes were extracted from the packing and solution with purified hexane. Control samples were attained by omitting the phosphoric acid in Packing No. 2. All investigations showed that undetectable quantities of aldehydes were extracted from the control samples by the lower bisulphite packing. The 2,4-dinitrophenylhydrazones were prepared on a Schwartz *et al.* (8) reaction column and the aldehyde 2,4-dinitrophenylhydrazones purified on adsorptive magnesia, according to the method of Schwartz *et al.* (9). The free aldehydes were obtained by regeneration with levulinic acid by the method of Keeney (4).

Gas chromatographic analyses were performed on a Barber-Colman Model 20 and also on a Research Specialties instrument. The Model 20 employed a 100-ft. capillary column coated with Apiezon L, a Tritium ionization chamber detector, and argon as the carrier gas. The Research Specialties was equipped with a 6-ft. stainless steel column containing a packing of 60-80 mesh, acid-washed Celite coated with Apiezon L. Strontium[90] was employed as the source for the ionization chamber detector and argon served as the carrier gas. The nature of the complex mixture necessitated varied column temperatures and pressures, in order to resolve and detect the individual aldehydes.

The straight-chain bound aldehydes of butteroil were identified by retention time, using authentic aldehydes as standards. The branched-chain saturated aldehydes were tentatively identified by plotting chain length vs. retention time on semilog paper.

A chromatographic analysis of the saturated aldehydes on the packed column revealed the presence of straight-chain aldehydes varying from nine to 18 carbons in chain length. In addition to the homologous series of straight-chain aldehydes, branched-chain aldehydes containing 11 to 18 carbons, with the exception of a C_{12} aldehyde, constitute a major portion of the bound carbonyls. A semilog plot of carbon chain vs. retention time (Figure 1) revealed that two types of branching were present in the complex mixture. This was further substantiated on the capillary column which separated the branched aldehydes containing 13, 15, and 16 carbons into two distinct peaks. Although not reporting the aldehydes bound in butteroil, Schogt *et al.* (7) proposed that the presence of aldehydes of less than 12 carbons in chain length is a result of autoxidation of the butteroil. The method

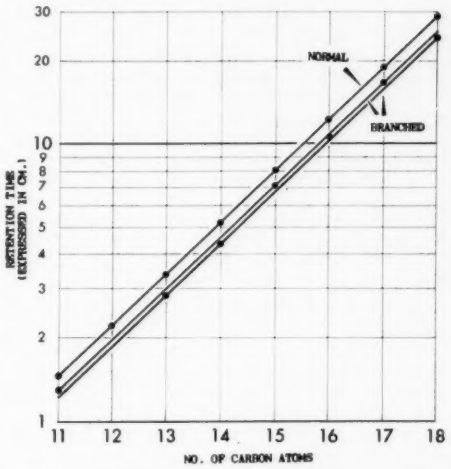

FIG. 1. Semilog plot of retention time vs. carbon chain of bound saturated aldehydes from butteroil.

reported herein compensates for any autoxidation and the results indicate that small amounts of C_9 to C_{11}, and possibly lower molecular weight normal and branched aldehydes, are bound in butteroil. A complete summary of the bound aldehydes detected in butteroil is presented in Table 1.

Direct reaction of butteroil with 2,4-dinitrophenylhydrazine also revealed the presence of small amounts of unsaturated aldehydes. The presence of three enals (approximate chain lengths of 12, 18, and 20 carbons) and three dienals (C_{16-20}) was established by paper chromatography, according to the method of Klein and de Jong (5), by ultraviolet studies in 95% ethanol, and by magnesia class separation (9). Smaller amounts of other enals ranging from 13-16 carbons in chain length also were observed.

TABLE 1

Summary of data on the bound, saturated aldehydes in butteroil and the plasmalogen fractions of milk

No. of carbon atoms	Butteroil			Plasmalogens		
	n-Aliphatic	Branched 1	Branched 2	n-Aliphatic	Branched 1	Branched 2
18	+	−	+	+	−	+
17	+	+	−	+	+	−
16	+	+	+	+	+	+
15	+	+	+	+	+	−
14	+	−	+	+	−	+
13	+	+	+	+	−	−
12	+	−	−	−	−	−
11	+	+	−	−	−	−
10	+	−	−	−	−	−
9	+	−	−	−	−	−

As a comparative study, the aldehyde 2,4-dinitrophenylhydrazones from the plasmalogens were obtained according to the procedure of van Duin (10), separated into classes on magnesia, and the saturated aldehydes regenerated and gas-chromatographed. Table 1, summarizing the saturated aldehydes from the plasmalogen fraction of butter serum, shows that the saturated aldehydes bound in this lipid fraction are similar to those in butteroil. However, the gas chromatograms in Figure 2 illustrate that the relative amounts of the higher molecular weight aldehydes (C_{13-18}) differ considerably within these fractions. Whereas the greater majority of bound, saturated aldehydes in butteroil range in chain length from 14 to 16 carbons, the C_{16-18} aldehydes dominate the bound, saturated aldehydes of the plasmalogens. Furthermore, the plasmalogens also contain a variety of unsaturated aldehydes, although still in relatively minor amounts. Evidence based on paper chromatography, behavior on magnesia, and ultraviolet studies indicate that enals of 16 to 18 carbons and eight to 12 carbons in chain length are dominant, with barely detectable amounts of C_{13-15} unsaturated aldehydes. Although all necessary precautions were observed to prevent autoxidation, the presence of the lower molecular weight unsaturated aldehydes as a result of chemical deterioration can not be disregarded.

The significance of bound aldehydes in the off-flavor development of various dairy products is under investigation at the present time.

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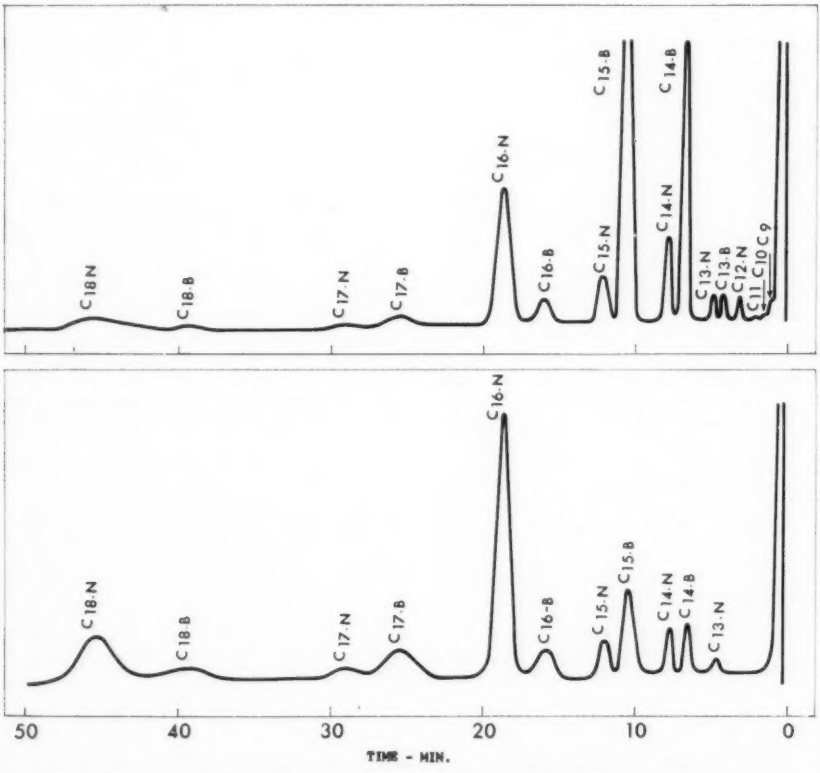

Fig. 2. Separation of bound saturated aldehydes from butteroil (top) and plasmalogens (bottom) on a 6-ft. stainless steel column containing 10% Apiezon L on 60-80 mesh Celite, temperature 175°, argon pressure—30 p.s.i., voltage—1,000. C_x = No. of carbon atoms; N = Normal; B = Branch.

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RELATIONSHIPS OF DATE OF CUTTING, STAGE OF MATURITY, AND DIGESTIBILITY OF ORCHARDGRASS [1, 2]

That forages in general decline in digestibility with advancing stage of maturity has been known for many years. Reid *et al.* (4) found the decline in dry matter digestibility of first-cutting forage to be related to date of cutting. They reported that the relationship between time of cutting in days elapsing after April 30 (X) until July 12 and the percentage of digestible dry matter (Y) can be predicted by the equation: $Y = 85.0 - 0.48X$.

Minson *et al.* (3) studied the digestibility of several grass species at different stages of maturity with sheep. They found that the percentage of digestible organic matter of first growths remained almost constant until the ears started to protrude from the leaf sheaths, then fell rapidly at approximately 0.5% per day. Cocksfoot was always less digestible than ryegrass at similar stages of growth.

Recently, experiments were initiated at this station to ascertain the relationships of cutting time, stage of maturity, and dry matter digestibility of forages in the Puget Sound area of Washington. This is a preliminary report of experiments conducted with orchardgrass (S-143) during 1960.

The green forage was harvested daily, beginning on April 22. The cut forage was fed fresh to four yearling heifers in individual feeding stalls. It was fed to appetite, but only to the extent that there would be no refusal. This prevented selective feeding.

Samples of forage were taken daily for dry matter analysis. Observations of stage of maturity and species counts were made weekly. Grab samples of fecal excreta were taken

TABLE 1

Relationships of dates of cutting, stages of growth, and dry matter digestibility of first-cutting orchardgrass in 1960

Date of cutting [a]	Stage of maturity	Average dry matter digestibility (%)
April 23		75.6
April 25	Vegetative	
April 27		75.0
May 1		75.3
May 2	Preboot	
May 5		73.9
May 9	Boot	72.2
May 13		67.3
May 16	Heads emerging	
May 17		63.7
May 21		60.8
May 23	Full head	
May 25		59.6
May 29		58.2
May 30	Early bloom	
June 2		56.4
June 6	Full bloom	54.8

[a] Cutting dates indicated for average dry matter digestibility were two days prior to dates of fecal collections.

[1] This study was supported in part by funds from the Dairy Cattle Research Branch under Cooperative Agreement 12-14-100-769 (52).

[2] Scientific Paper No. 2133 Washington Agricultural Experiment Station, Pullman. Project No. 1333.

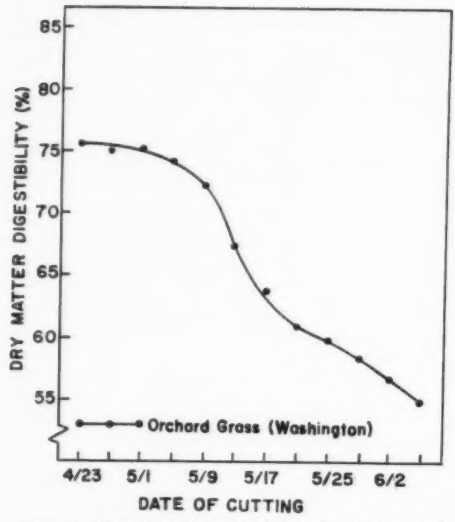

FIG. 1. Relationships of date of cutting and dry matter digestibility of forages.

Since it is assumed that approximately two days elapsed between the time of forage ingestion and excretion of the undigested portion of that forage, the cutting dates indicated for digestibility measurements in Table 1 and Figure 1 are two days in advance of dates in which fecal collections were made.

By comparing Table 1 and Figure 1, it is evident that the most rapid decline in dry matter digestibility occurs while the grass is advancing from the boot to the head stages.

Table 2 shows the sum of squares owing to fitting polynominals through the sixth degree to days, according to the methods of Fisher and Yates (2). As expected, the linear component accounts for the majority of the variations in days. However, the sum of squares accounted for by the third and fifth degree polynominals are significant at the 0.0005 (or less) level of significance. Thus, it is concluded that the line can best be represented by a curve.

Digestibility data herein reported are also being related to various chemical analyses, as well as in vitro and in vivo digestion methods. Results of this phase of the work will be reported later.

TABLE 2

Analysis of variance of per cent of dry matter digestibility of orchardgrass in 1960, with the levels of probabilities for the day components

Source	df	SS	MS
Cows	3	2.69	0.90
Days	11	2,861.93	
1st Degree	1	2,738.85	2,738.85 ($P < 0.0005$)
2nd Degree	1	4.82	4.82 ($P < 0.05$)
3rd Degree	1	85.01	85.01 ($P < 0.0005$)
4th Degree	1	7.14	7.14 ($P < 0.025$)
5th Degree	1	16.59	16.59 ($P < 0.0005$)
6th Degree	1	4.97	4.97 ($P < 0.05$)
Residual	5	4.55	0.91
Error (Cow × day)	33	20.55	0.62

from each heifer at four-day intervals, beginning April 25. Samples representing the first day's collection and subsequent ones at four-day intervals were extracted for chromogens, according to the method of Brisson and Hatina (1). Digestibility of forage dry matter was determined from the chromogen analyses, as suggested by Reid *et al.* (5).

Although the forage stand was predominantly orchardgrass, some bluegrass and weeds were present. The average composition of the stand on a dry weight basis was 75% orchardgrass, 22% bluegrass, and 3% weeds.

The average digestibilities of the dry matter of first-cutting orchardgrass by successive four-day intervals are shown in Table 1. The stages of maturity of the forage at weekly intervals are indicated in the same table. The curvilinear relationships of dates of cutting and dry matter digestibilities are shown in Figure 1.

ACKNOWLEDGMENT

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INHIBITION OF RUMEN CELLULASE BY AN EXTRACT FROM SERICEA FORAGE

The nutritive qualities of sericea (*Lespedeza cuneata*) forage for dairy animals are generally considered to be inferior to those of other crops, such as alfalfa (5). Although low intake and inferior quality of sericea forage have been associated with the high tannin and lignin content, chemical tests for these constituents are not entirely satisfactory as a means of predicting the value of the forage.

Recently it has been shown (1) that Muscadine grape leaves contain a water-soluble substance which inhibits the enzymatic hydrolysis of soluble cellulose. The substance was stable to heat, to weak acid or alkali, and was nondialyzable through cellophane against water. The cellulase inhibitor of grape leaves has been suggested to be a tannin-like substance, and such materials have been associated with the inhibition of certain hydrolytic enzyme systems such as pectinase (4). In view of the high tannin content of sericea, it was decided to test a water-soluble extract of this plant for a possible inhibitory effect on rumen cellulase activity.

EXPERIMENTAL PROCEDURE

Leaves were collected in October, 1960, from mature sericea (seed stage) grown at the Central Crops Research Station of North Carolina State College, Clayton, North Carolina. An extract was prepared by blending 15 g. of fresh sericea leaves with 300 ml. of distilled water for 10 min. The slurry was pressed through two thicknesses of cheese cloth and the aqueous extract was centrifuged for 15 min. at 3,000 r.p.m. The clear, straw-colored portion was removed and either tested immediately for effect on cellulase activity or frozen for later testing. Rumen fluid obtained from a fistulated bovine steer of the North Carolina State College herd was used as the source of cellulase. The rumen fluid was filtered through cheese cloth, centrifuged at a speed of 3,000 r.p.m., and mixed with distilled water to make a 20% solution. The prepared fluid was either used immediately or kept frozen until needed.

The viscometric method described by Bell *et al.* (1, 2) was used. Enzyme activity was expressed in cellulase units: 100 units equal 50% loss in viscosity of a 1.0% solution of sodium carboxymethylcellulose (CMC-7M, Hercules Powder Company, Wilmington, Delaware) buffered at pH 5.5, and held at 30.0° C. for 20 hr. Standard enzyme-reaction curves were used to convert percentage loss in viscosity to units of activity. Four milliliters of the 20% rumen fluid were mixed with 2 ml. of water extract of sericea. Distilled water was used in place of the inhibitor and/or enzyme solutions for the controls. The optimum pH for rumen cellulase was found to be slightly above 5.5, with 1% CMC at 30.0° C.

Fresh leaves of fescue (*Festuca arundinacea*), ryegrass (*Lolium multiflorum*), alfalfa (*Medicago sativa*), and soybean (*Glycine max*) were also extracted and tested as described for sericea.

RESULTS AND DISCUSSION

Sericea extracts had an inhibitory effect on rumen cellulase activity, as shown in Table 1,

TABLE 1
Effect of sericea extract and heat on 20% rumen cellulase

	Cellulase units [a]	Cellulase inactivation
		(%)
Rumen fluid	255	
Rumen fluid, heated	3	98
Rumen fluid plus sericea extract	3	98

[a] Each value is the mean of two replications.

whereas the other forages tested had no marked effect. Effects of various dilutions of the sericea extract were studied and are presented in Table 2.

These in vitro observations show that sericea water extracts contain a compound or compounds which will inhibit hydrolysis of carboxymethylcellulose. This compound has not

TABLE 2
Effect of increasing levels of sericea extract on rumen cellulase activity [a]

Sericea extract	Cellulase inactivation
(%)	
5.00	96
1.00	87
0.50	75
0.10	42
0.05	30
0.001	18

[a] Each value is the mean of two replications.

been isolated or identified, but a color test for polyphenols and the suppression of the inhibition by polyphenol oxidase from the mushroom indicate that it is polyphenolic in nature. It is nondialyzable through a cellophane membrane against water and is unstable in alkaline solutions. The effects of this compound (or compounds) in in vivo digestion have not been determined, but it has been suggested by Gill and King (3) that there may be rumen cellulase inhibitors and that information on their nature, mode of action, and origin might lead to improved digestive efficiency of ruminants. This will be tested in vivo with sericea breeding lines which have been shown to contain varying concentrations of inhibitor.

SUMMARY

(1) A water-soluble substance or substances in the leaves of sericea lespedeza, which inhibits the enzymatic hydrolysis of soluble cellulose, is reported. The reduction in cellulase activity obtained was proportional to the concentration of the inhibitor present.

(2) Extracts of fescue, ryegrass, alfalfa, and soybean forages were not inhibitory to rumen cellulase.

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SYMPOSIUM: SELECTING A CAREER IN THE DAIRY INDUSTRY[1]

CAREERS IN EDUCATION AND RESEARCH

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TO DELINEATE THE TOPIC

In our consideration of Education and Research as a career in the dairy industry, it would seem best that attention be directed only to that work in a university, college, or department which offers studies in dairying.

It must be recognized that some educational, or teaching, duties are involved in nearly all careers which a dairy major may contemplate. Some students in dairy production anticipate work in vocational agriculture. But here, no research duties are involved; we shall not consider this field of education. Extension work revolves almost exclusively around educational, or teaching, duties with farmers or dairy processors, along with many activities of a demonstrational nature, but seldom is a worker in this career in dairying able to devote much attention to formal research projects. Thus Extension, which is an excellent and satisfying type of work, would seem beyond the scope of my topic.

Furthermore, a dairy-trained man in Foreign Service or in dairy production or manufacturing often confronts situations that involve teaching, but these are contemplated under other topics of our program today.

Finally, a career in dairy production or in dairy manufacturing, as we shall be told later, offers a multiplicity of specific choices. Among these is research work for some commercial concern. This can be highly fruitful and rewarding for the properly trained dairy student, but, again, it is considered beyond the scope of my assigned topic to delve into these opportunities in so-called commercial research.

IN CONFIRMATION OF OTHERS

As you will have heard several times before this program today is ended, we older men are gratified to meet here with the young men—and the young women—who obviously are interested in the over-all topic of the symposium. The pleasure I find arises from the realization that most of you are devoting yourselves to our industry; you have manifested your interest in it; some of your experiences and observations have attracted you to it; you are quite committed to some career in dairying as your life work.

For these reasons I offer congratulations. With complete confidence, I can predict that the majority of you will never have occasion to regret your identity with our industry and that you will continue this identity over the years.

It is recognized, too, that as opportunities arise for each of you in the next few weeks, or months, or even years, and as your plans materialize, some of you will reach a decision to embark on a career far removed from the dairy industry. Often we proud dairy professors convey an attitude of distress when we learn of a man who is trained in dairying departing from the industry. Actually, we are wrong in bemoaning such changes. The dairy industry enjoys no monopoly on career opportunities; the dairy training a student has in his university, the experience he will have secured, all can contribute to success in his newly selected career.

Dr. Trout in the JOURNAL OF DAIRY SCIENCE, 41: 12. 1958, has made a report on the careers of 1,401 men who participated in the International Dairy Products Judging Contests from 1930 until 1958. Seventy-four per cent of these were at work in some phase of dairying. Nineteen per cent were in work unrelated to dairying. This included men who were in military service. There was 7% whose addresses were unknown or who were deceased. By far the greater number of these judging contestants found the dairy industry to their liking.

My two sons majored in Dairy Manufactures. One who graduated in 1943, after two years in military service, started in dairy work and is still doing sales work with dairy equipment and supplies. He and his wife and three children are happy and content; so am I. The other son, who graduated in 1949, remained in dairy work for three or four years. He was always intrigued with accounting; he secured a Master's in that work, is now a CPA, and works with an accounting firm. He and his wife and three children are happy and content; so am I. I am unable always to express regrets when a dairy major abandons his dairy training—in part—and enters some other career.

A CAREER IN EDUCATION AND RESEARCH

In more than 40 years of consultations, counselling, and advisory service with dairy undergraduates, I've encountered many who, even in their senior year, were not too certain as to the particular dairy work in which they defi-

[1] Presented at 56th Annual Meeting by Student Branch, A.D.S.A., Madison, Wisconsin, June 13, 1961.

nitely expected to engage. Again, I can not consider this situation particularly unfortunate, although it can be frustrating. These students were realistic; they—as well as I—were aware that many circumstances, often unexpected, can arise to dictate a decision; they had to weigh all aspects of the situation they might be encountering on graduation.

On the other hand, I've found many students who had their objectives fixed firmly for their careers. The selected careers always vary from student to student. Some have no thought other than to farm. Their numbers have been declining over the years. Other students with established goals for careers have intended to do work in plant operation and supervision; some plan to engage in sales; others in personnel service where they can exercise talents to work with people; many have had definite plans for graduate work. In recent years, it is evident that more of our undergraduate students have made their plans and selected their courses to qualify them for research work. Most frequently, the man who enters research work at an experiment station also has duties on the teaching staff.

I have worked with hundreds of these undergraduates who have had quite definite career goals, which they indicated to me. But here is a potent observation: among all these students the number who have indicated a desire to become university teachers is exceedingly low. I'm sure I could count them on my fingers. Far more often, these dairy students I have known have avowed they did not desire or plan to become teachers. And more often yet, I've pondered the reasons for this antipathy.

Despite the early lack of desire by dairy students to become university teachers, many change their minds later. The study by Doctor Trout, just mentioned, reveals that 6%, or 83, of the contestants in dairy products judging became university professors. At the time the study was made, 60 of the contestants were engaged in graduate work; doubtless, many of these by now have entered the teaching profession.

However, here it is suggested that these figures for judging contestants in dairy products may be higher than comparable figures for all dairy undergraduates.

SOME UNFAVORABLE FACTORS

In my advisory work and career counselling over the years, I have always resisted the tendency to oversell the dairy curriculum or dairy opportunities to high-school graduates or to underclassmen attempting the decision on their major. Such overselling is neither necessary nor desirable; it can prove unfortunate.

By the same token, I intend here, on this symposium, to avoid undue propaganda for a dairy career in Education and Research. It's best to call attention to the handicaps and limitations, as well as to the advantages and opportunities.

There are some six or seven handicaps or limitations which often occur to the dairy undergraduate. I would enumerate:

1. Employment in Education and Research virtually requires nowadays that a man have the Doctor's degree. This means three years of graduate study. But few professions—notably medicine—require more formal studies than that of university professor.

Actually, of course, this additional training for dairy students to become teachers and research workers should not be considered a handicap; it's an opportunity, just like your four years of undergraduate study have not been a handicap but an opportunity.

2. The costs for the graduate study can be considerable. This situation can be aggravated by the fact that the man's professional earnings are delayed or interrupted during the period of graduate studies.

Attractive scholarships and fellowships are available to minimize costs in graduate study. However, the holder of one of these may be delayed for even another year or two in completing his graduate study.

Many undergraduates in their contemplations conclude that the costs in graduate study are prohibitive for them.

3. An opinion prevails that salaries for university professors are appallingly low. I'm not unhappy that this opinion prevails; however, I am forced to assert it's not true. I face ostracism by my colleagues in these assertions, but that's not too serious; I'll be a university professor for only 382 more days, anyhow, before I'm retired on July 1 next year.

On the average, the men who are engaged today in Dairy Education and Research are receiving salaries quite commensurate with those in most other professions. To be sure, some men in other work receive salaries which to us seem fabulous; likewise, others in the same work are on a pretty low scale. Most professions have a wider salary range than universities.

4. University students often have an image of the university professor which is not too flattering. I agree with the students although, fortunately, some professors are not typical—but exceptional and good.

5. Dairy undergraduates can obtain a viewpoint that teaching work is drudgery—with lectures, demonstrations, and laboratories to conduct, reports to maintain, the necessity to evaluate student achievements, grading papers.

6. Most university professors are subjected more or less frequently to criticism from students in their selection of subject matter, teaching methods, preparation of examination questions, grading. Most such criticism is ridiculous; dairy professors with devotion to their work and with experience usually know far better

than any student how to teach a subject. But students think they know; nowadays, more than in years past, they are growing more vociferous; the teacher takes unwarranted abuse. Many students who witness this situation avow they never want to be subjected to such ordeals.

7. In respect to dairy research, some students look upon it as a drab, confining, unrewarding occupation.

REWARDS IN THIS CAREER

It was 44 years ago next September that I took my first position as a dairy teacher at the University of Minnesota. Subsequently, I've worked in three other universities. I've undergone all the tribulations and ordeals just mentioned, but never for a moment have I regretted that I was engaged in Dairy Education and Research. The rewards have far outweighed the trials. And I'm sure there are dozens of my contemporaries and hundreds of younger men in this work who can submit the same testimony.

Here, I am not intimating you should all contemplate this career. Most of you can and will achieve notable successes in other phases of our industry. But for all of you, I'd like to enumerate the rewards that can be anticipated in Education and Research.

1. Average salaries are adequate.

Ethically, it might be preferable that a topic so mundane as salary not be mentioned first, but I shall not apologize. I'll not even propose it be considered of lesser importance.

For a man of my limited abilities, and in view of the fact the services I have rendered have been only moderately effective, the monetary rewards have been highly satisfactory, at least to me. I'm not sure how my employers may have felt. Maybe some other phase of our industry could have afforded me greater return. I don't know what other phase it could have been.

2. Education and Research work in a university offers a high degree of security. Although I would hope that such is not true, it is possible this job security may lead some staff members into a state of lethargy toward their work; they may tend to coast.

3. A university professor seldom is under the pressures and demands for results that are the bane of workers in some other phase of our industry. The fierce competition of industry is not encountered.

4. Unequalled social and cultural opportunities characterize the lives of university professors and their families.

5. By nature, workers in Education and Research in our industry are adept in maintaining contacts with farmers, breeders, plant owners, operators, and dairy organizations, and in serving the individuals and groups.

6. Rewarding experiences in these contacts and services are inevitable. They are useful in the faculty members' teaching and research.

7. No man in any career can enjoy a greater reward than can a dairy research worker who, by application of known scientific principles, can evolve procedures to avoid problems and improve practices in dairy production, processing, or distribution.

8. Effective professors find untold pleasures in the opportunity to work with students, to guide them in their development, to arouse their interests in obtaining further knowledge, to help advance these students in their careers.

9. And the culminating reward of all, in Education and Research, is to observe the quite universal successes of the former students who now are co-workers, as they progress in their careers in the dairy industry.

CAREERS IN DAIRY MANUFACTURING

H. F. Judkins

Secretary-Treasurer, American Dairy Science Association, White Plains, New York

While there are other opportunities in the dairy manufacturing field, I will confine my comments to those having to do with dairy processing.

I. Areas of opportunity in dairy processing are as follows:

1. Procurement of dairy raw materials.
2. Purchasing of supplies and equipment.
3. Plant supervision.
4. Research and quality control.
5. Engineering.
6. Transportation.
7. Accounting.
8. Insurance.
9. Taxes.
10. Legal.
11. Personnel.
12. Public relations.
13. Sales.
14. Executive Management.

II. Is your college training opening the doors for opportunity?

In the processing division one needs training in dairy technology, with emphasis on the sciences for quality control and research and on business administration for company operation.

One hears a good deal about the curriculum for the dairy technology student. While I would not belittle its importance, I feel it is not nearly as important as the type of training the graduate gets after he enters business and how well he applies himself to a continued program of education. There is abundant proof of this when one considers the type of training that many successful men in the dairy business received while they were in the college or university. Some of the older successful college men now in the dairy business took a very general course in college; in fact, about everything that was offered. This curriculum was much different from what many would consider adequate today, but by application they became very successful.

There are a number of earmarks of the college-educated man that are not measured on report cards. A man is seemingly born with a certain amount of the elements that involve what we call personality. The university should do everything possible to develop these personality traits. The teacher should stimulate the student to do his best and more than he has to do to get by.

Every student, regardless of his major choice, should be given plenty of training in oral and written English. The lack of ability to sell oneself and the work one is doing is the major reason some people do not attain important positions.

A college student has ample opportunity to learn to get along with all types of individuals and he should make the most of this opportunity. In carrying on a busy program, both in the classroom and in extracurricular activities, the student has an opportunity to learn to plan his work and work his plan, to make the best use of his time. In later life, this will be found extremely important.

Another very important success essential that a man should start developing in college is the ability to make prompt decisions. Decision-making in business on the part of men in key positions may represent the difference between a profitable and unprofitable business.

III. On-the-job qualifications: In addition to the basic qualifications just mentioned, the dairy business requires:

1. Men with good health.
2. Clean habits. The man who is slovenly about his appearance has no place in a dairy plant.
3. Promptness and regularity. No plant can operate efficiently unless employees report to the job on time. Absenteeism for any avoidable reason can not be tolerated.
4. Job interest. A man must be extremely interested in his job and do more than he has to to get by, and at odd times learn something about the jobs that lie ahead.
5. Cooperativeness. For smooth-running organization a man needs to have a happy disposition, get along well with his fellow workers, and be willing to do his share of the work.
6. Why and how. A man must know why as well as how his job should be done and as a supervisor be able to teach others.
7. Handling people. As a supervisor, one must be able to handle people in a way that they will work for him, look up to him, and respect him.

It is a fact that dairy companies, in general, have been woefully weak in handling and training college graduates. To help improve this situation, the American Dairy Science Association published a Dairy Industry Plant Training Manual about a year and one-half ago. Over 1,500 of these manuals have been sold to industry. Time prevents discussing this book in detail, except to say that if every one entering the dairy processing business makes all the studies and answers all the questions listed in this manual, he can not help having a more interesting and successful life. Every graduate entering the dairy processing business should purchase one of these manuals and whether or not the company with which he is connected uses the manual in installing a training program, the graduate by applying himself can do much to train himself by using this manual.

IV. Advantages of the dairy industry for a life career. Among the many advantages of the dairy industry for a life's career are the following:

1. The industry provides steady employment.
2. The dairy industry is so widespread that one can work in it and live almost anywhere, in the country he chooses.
3. There is satisfaction to be gained in knowing one is working for the public good, since dairy products are so essential and so universally used.
4. The industry offers an infinite variety of work, all of which makes life very interesting.

V. Remuneration and chance for advancement.

Starting salaries in the commercial dairy business are equal to those in other fields, with the possible exception of engineering. It is quite possible that key positions in the dairy industry, at least below those of top management, do not pay as high an annual salary as some of those operating on a higher percentage

of profit, and the defense industries. It must be kept in mind that in the dairy business, as well as in other businesses, it generally takes time to attain a key position. A survey that I made just a few years ago in a number of companies showed that the time from start to what might be called a key position averaged about twenty years. Of course, there are certain things, such as marrying the president's daughter, that can alter this, but these cases are the exception rather than the rule. Patience is a great virtue and one must have it.

VI. Outlook for the future.

The trend is toward fewer but larger units, which are going to require men of high caliber to operate. Population is increasing steadily. This means ever-increasing consumption of dairy products. It can truthfully be said that the future never looked brighter.

CAREERS IN DAIRY PRODUCTION

H. A. HERMAN, Executive Secretary
National Association of Artificial Breeders, Columbia, Missouri

It is a pleasure to address the Student Section of the American Dairy Science Association and discuss briefly with you Careers in Dairy Production. There is no subject closer to my heart. As a dairy farmer and cattle breeder, as a professor of Dairy Production for nearly 25 years, and with some eight years of experience in my present position, I have formed some very definite opinions regarding the future. The ideas I present are gathered from experience and observation over the entire dairy production field.

FUTURE NEVER MORE CHALLENGING

In my opinion, the future for a worthwhile career in dairy production was never more promising. At the same time, I am sure the man who is successful in this field in the future must be better prepared from the standpoint of knowledge, judgment, business sense, and courage than the dairy production major who graduated twenty years ago. This is largely because of the tremendous changes in our agricultural picture in the past twenty years. The dairy production picture has changed a great deal.

As you know, in 1945 we had nearly five million farmers. Now we have only about three and one-third million. We had well over one million dairy farms selling milk or cream—now we have only about 985,000. Our average farm is larger by 100 acres. We have 25% less milk cows and some 25% fewer dairy farmers. In 1916, our average DHIA-tested herd was 16 cows. Now it is 44 head and still growing.

The producer-distributor has almost disappeared. So have many of our small creameries. Our local cooperative creameries so vital to the dairy farmer 20 to 30 years ago have merged, joined with, or sold out, to a national dairy operation, or have folded up. In this milk business we have developed volume operations that result in the cheapest and best bottle of milk in the world, delivered to our consuming public.

We have moved into an area where service and selling, as well as producing and processing, play a vital part. At the same time, we have increased emphasis upon a quality product. In the milk-producing business we would not want the quality picture otherwise. We also have come to recognize the dairy business, both production and products fields, as part of a highly developed and highly competitive field. It is a business that requires more capital investment per worker than any phase of agriculture and, as you know, the investment per average farm worker is $27,000 and in industry it is only about $15,000 per man. I can cite you successful dairy production operations where the investment per worker is in the area of $30,000 to $50,000 per man—land, cattle, buildings, equipment, etc. Dairymen who recognize this challenge and meet it are making a living and a fair return on the investment. Any man who desires a career in dairy production can succeed if he has the correct perspective of the job ahead.

I should be the last person in the world to criticize our teaching in agricultural colleges, but I do not hesitate to say that in my opinion **there is often too much attention and time spent on minute details and the student never gets a glimpse of the broad picture he faces when he graduates.** Frankly, I feel that any student who wishes to succeed in this field should bury his diploma good and deep, not haggle too much about starting salary and time off, and get a few years of good hard-headed experience—if he hasn't had it before—and then start to build his career. He then will find his college training invaluable and, along with good work habits, his career will begin to unfold.

WHAT ARE SOME OF THE JOBS?

Time and space will not permit my listing any but a few key jobs in a few fields.

Keep in mind that as a rule jobs go to those best prepared to fill them! By the same token in merchandising—even our artificial insemination of cattle program of which I am very proud—"markets go to those best prepared to serve them."

1. DAIRY FARMING AND CATTLE BREEDING.

For the dairy production major, the love of cattle and of the land is usually very strong. Even though he finds himself working for a salary, he dreams of the day he can own his farm and his herd. This goal is attained sometimes, but not too often, for the simple reason it takes so much money to start dairy farming the average man, after paying income tax, supporting a family, owning a home and a car, hasn't much left.

(a) The best way today to begin, for a young man who wants to be a dairy farmer, is to go right back home and go into partnership with his father and put his ambition and knowledge to work.

(b) Another plan is to go into partnership with an old, established dairy farmer and gradually buy into the setup. Most of our dairy farmers are past 50 years old. Some very good herd owners are looking for the young man who loves cattle and the soil, to team up with.

Such jobs usually start with the young man acting as assistant manager. If he has the stuff he gradually becomes part-owner. There are more opportunities along this line than many people appreciate. Just consider the established herds in your own community. Who will carry on when the present owner no longer can "hit the ball"?

(c) Along with most established herds is the purebred cattle challenge and the pleasure of producing some seed stock, as well as milk. Today, we have too few constructive breeders. We need in our A.I. program more proved sires, more good cow families and, industrywise, more good cattle.

Due to our present income tax laws, and our farm labor picture, there is a declining number, and will continue to be, of wealthy herd-owner hobbyists. These men, by virtue of sufficient capital, assembled many great animals. They bred some of our best cattle. They also purchased some of the top cattle from smaller breeders. In the future, farm-sized herds and the semi-commercial herd will, by virtue of a sound breeding and testing program, produce much of our seed stock for the future. The farm-sized herd, where some individual cow attention can be given, will continue to be the leader in this area.

Going into business for himself will not for a long time have the glitter of a salary job. New cars, new suits, and a few of those things won't come too fast. An understanding wife is a must in this picture. Most fellows who choose this course won't be quitting at 5 P.M. and come in smelling like a rose for a 6 P.M. dinner. In the long run, however, they will amass more total capital, have more independence, more freedom of action and thought, be healthier, have a closer-knit family life, be more appreciated in the community, and have less heart trouble than the classmate who chooses the humdrum city life and keeping up with the Joneses.

2. HERD MANAGEMENT—FARM SUPERINTENDENT.

It has long been said that "If you can't own your own herd the next best thing is to manage a good herd and farm for someone else." This is true today—but different than 25 years ago. There are fewer farms supported by some other business. Most farms today have to make money or at least pay interest on the investment. In America, there are vastly fewer jobs of estate managers than we once knew. There will continue to be less! The hired dairy farm manager today will have to:

(a) Possess good business judgment and be able to handle large capital and to direct employees.

(b) He will have to be an expert dairyman, as well as a farmer, and produce milk in large volume at low cost to meet competitive market demands.

(c) He will have to possess ingenuity and exert energy to keep his operation right-side-up in the face of the changing agricultural picture. He will also have to be a good salesman, to insure his employees and his stockholders that the future is sound and that his operations are equally sound. When one works for a Board or a corporation, he must always be prepared to defend his position.

It is my observation that to some extent good cow men and men capable of large-scale dairy operations are born with much of the potential. I have never believed it can be taught except by experience. I do believe sound teaching plus good experience can develop many men along these lines.

In the years ahead I forsee an acute shortage of capable managers who can handle a $500,000 to a two million-dollar dairy production setup. This is what we must face, because we will have many more 200 to 2,500 cow herds, with the resulting capital, labor, feed, disease, and marketing problems. Men who can handle these jobs will always be few, but well paid. They should be well paid, as they are virtually, in the dairy field, what the economists call captains of industry.

3. DAIRY PROCUREMENT AND FIELD WORK.

There will continue to be increasing emphasis upon much procurement. As our dairy processes become fewer, with greater volume, greater overhead expenses, and tougher competition the importance of milk procurement increases. Plants are constructed to handle milk. They cost a lot of money. Without sufficient volume they quickly become a liability.

Procurement work involves direction of field staff, quality control, truck routes, and a score of problems that challenge the best of every worker in this field. It requires from the director to the newest fieldman a thorough understanding and appreciation of the problems of the producer and willingness to cooperate with him.

4. CATTLE IMPROVEMENT—ARTIFICIAL INSEMINATION.

(a) Our breed registry organizations are constantly adding men to serve as editors, advertising representatives, and field representatives in charge of registration and production testing. It is a good field and offers an opportunity to work with people and cattle.

(b) Artificial insemination—This field is new. Starting just 21 years ago, the program now embraces over seven million, or 37%, of our milk cows in America. There are roughly 12,000 employees in this field at present, but the number increases daily. Here are a few of the key jobs and their requirements:

General manager. Requires a man capable of directing from 50 to 1,000 employees and heading a business with gross returns of $300,000 to as much as five million dollars a year. General Managers must be interested in cattle improvement, understand business management and finance, be good personnel directors, and be able to carry on a strong public relations program.

Assistant manager. Assists the manager in his varied duties. Requires same type of experience and training. Offers advancement to full managership.

Field supervisor. In charge of field operations. Includes supervision and working with technicians, selling the program, public relations and, in some cases, trouble shooting. This is a type of extension work in many ways. Large organizations have a Director of Field Service, who directs the work of many field supervisors. Love of cattle and ability to supervise personnel is a must in this position.

Sire analyst. Every organization has a sire analyst, whose duties are to constantly evaluate sires in use and comb the field for improved sires that will improve the patron's herds. One or more assistants may also be employed. This job requires a basic understanding of animal genetics, working knowledge of breed families, ability to analyze records, evaluate cattle for conformation, etc., and prepare copy for advertising sires in use.

Laboratory supervisor. This man must be scientifically trained. He has charge of semen collection, processing, storage, and shipping. His position is similar to that of Production Manager in many industries. A key job and one of the most vital. A man in this position must be research-minded, because he is constantly ferreting out weaknesses in his organization's operations that influence settling rate in the field.

Technician or inseminator. While mentioned last, by no means is the technician, whose job it is to inseminate cows, the least important in this field. In most cases he is the key man. He is usually well paid—often better than most of the positions listed above—if he is in a heavily populated cow area and wants to work. Many technicians are college graduates, though the majority are not.

Working as a technician for a year or two is vital to the success of men in all positions in A.I. work. This is the place a college graduate should start his training for a more advanced position in this field.

Other jobs. Most organizations publish a monthly or quarterly pamphlet, sent to all members. A part-time, or in some cases a full-time, editor is employed. He also assists with publicity, sire catalogs, advertising, and public relations. Here is the field for the dairy production man who likes to write.

The National Association of Artificial Breeders is the over-all organization combining the A.I. organizations in America. It also has members in Canada and numerous foreign countries. This organization is set up to carry on education, research, promotion, public relations, and aid in regulation of the program. Positions on the NAAB Staff include Executive Secretary, Assistant Secretary, Advertising Manager, Editor, etc.

5. TEACHING—EXTENSION—AND AGRICULTURAL EXPERIMENT STATION RESEARCH.

The immense opportunities for service to the entire dairy production field through teaching, and by extension work in all of its phases, is known to most agricultural college graduates. It is a worthy field. **Men who expect to teach Dairy Production should be well fortified by actual experience in the production field and have a broad perspective of the field, in addition to the usual textbook knowledge.**

Research is vital to our continued progress. Our research men today must have much more

fundamental science than was true 30 to 40 years ago. The opportunities are great and the young man who expects to enter the research field would do well to take considerable post-graduate work in basic biological, physiological, and physical sciences.

6. INDUSTRY

The dairy industry claims most of our production graduates. The openings are too numerous to mention. Fundamentally, the ability to sell, advertise, write, and speak well are assets for all except key research men in these fields concerned with merchandising our nearly 130 billion pounds of milk a year. Technically trained men in quality control work, dairy and farm equipment, plant foods, insecticides, building materials are used throughout the industry.

I believe the above statements point out the vast opportunities the dairy production field offers the young man today. As I see it, **one of the most important needs for any young man is perspective of the field he enters.** Know where your career might lead! It doesn't make too much difference where you start in life. It's where you end up that counts. Success is not necessarily just making money, but rendering worthwhile service and making contributions to the welfare of our society.

It has been said "No trail is too rough when vision sees a worthwhile goal." Also, remember that most worthwhile things come from "about 1% inspiration and 99% perspiration." I have the greatest confidence in our young men entering the dairy production field today and I feel our future is in good hands!

CAREERS IN DAIRY INDUSTRY'S FOREIGN FIELD

DAVID L. HUME

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Last May 17, President Kennedy was in Ottawa, Canada; Vice-President Johnson was in Bangkok, Thailand; and Secretary of State Rusk was in Geneva, Switzerland. On this single day our three highest-ranking government officials were in widely separated foreign countries located on three continents.

How does this information relate to selecting a career in the dairy industry? I think it illustrates several important things which will affect your careers, inescapably, regardless of whether you remain right in your own back yards or locate in the remote foothills of some far-off outpost halfway around the world.

The importance of foreign service—its relatively new importance, too—is reflected by the fact that it takes our three top leaders of this nation outside of the United States on the same day!

Distances have been reduced by the almost unbelievably accelerated time in which communication and travel can now be accomplished. Despite the distances that separated our top governmental executives last May 17, they were less than 24 hr. apart in terms of time, and only minutes apart on the basis of speaking to each other. Calculated time-wise, all were closer to Washington, D. C., than President Calvin Coolidge was in 1927 when he vacationed in the Black Hills of South Dakota, and President George Washington when he visited New York City.

I would suggest that the compression of distances and time—afforded by modern travel and other forms of communication—will draw the world dairy community closer and closer together. You will, therefore, make more personal friends in foreign lands than was possible for your teachers, and you will frequently see and speak face-to-face with those new friends, as they visit you and you them. I would forecast that in larger and larger measure your careers will consist of exchanging views with our foreign dairy friends. You will be contrasting the specialized dairy problems of your work with those of their work, and this will be true in any phase of the broad range of activities in the dairy field.

As time passes, you will, I suggest, be in a continuous state of adjusting to a greatly expanding environment, in which international contact—as for many it already is today—will become a necessary routine of any successful professional career in the dairy complex. This does not mean that you will necessarily live and work in a foreign country. But it does mean that wherever the future dairy careerist elects to serve, contact with the foreign field will be inescapable in the world in which he will live. To paraphrase the point: In one sense you will not need to select a career in the foreign field; the foreign field has already selected you.

Sooner or later, however, some of you will serve dairying by living and working in foreign lands. In many countries, and in most of the large cities of the world, such service can be comfortable and interesting. In seeking foreign service there are, however, several factors you should consider.

Frequently, knowledge of some language besides English is required. Then, too, it is the usual thing that career people in foreign endeavors, not only in governmental service but in business, research, and among the international agencies, are required to move around

a great deal. So you will live among strangers, and often in an environment with standards of sanitation and living vastly different from those in the United States. These are factors to be considered with, or in the anticipation of, a wife and family.

While some jobs pay very well, salaries, generally speaking, are adjusted, so that the standard of living will be about the same anywhere in the world. In other words, with minor exceptions, going overseas to work is no assurance that you'll earn a lot of extra money.

Believe it or not, most often the experience you get working overseas is discounted when you get back to the United States and attempt to relocate in a new job. While this is not always true, it certainly is no exception. I have been told by those who have served overseas that it is extremely difficult to find any premium in obtaining a domestic position by reason of having had overseas service.

Foreign service sometimes requires you to be separated from your family for long periods of time.

On the other side of the coin, there are compensations.

Foreign work and living in foreign countries demand of you a broader interest in political, social, and economic conditions. If you like to learn and become aware of these things, foreign service will bring you in contact with this kind of activity.

Most of us enjoy foreign travel and study. A career in the foreign field is one approach to fulfilling this kind of enjoyment.

The sheer pleasure of learning and observing the way other people live and participate in their social and governmental institutions can be a very rewarding experience in itself.

Virtually all of us can derive some satisfaction from the sense of having done a good job of representing America abroad. You do not need to work for the government to do a good job of representing the United States. Any of us who goes abroad is, in fact, a representative of his country. Doing a good job of this, by living and working abroad, gives a sense of satisfaction to most people.

Usually, foreign service provides a little more liberality as far as vacation is concerned. And, despite the fact that pay is sometimes low, an expense account including some allowance for quarters usually accompanies most jobs, whether commercial, private, or governmental. These are only a few of the things you should think about before selecting the field of foreign service for a career.

Epictetus, a Stoic philosopher, has been quoted as saying: "No great thing is created suddenly, any more than a bunch of grapes or a fig. If you tell me that you desire a fig, I answer you that there must be time. Let it first blossom, then bear fruit, then ripen."

I ask you to ponder the meaning of this quotation as I proceed to the final phase of these remarks.

I have written to several of our dairy leaders serving in several parts of the globe in several phases of dairy work. They have responded generously to my concern for helping you consider a career in the foreign field. Almost unanimously, in one way or another, I have been charged to tell you not to force your way into foreign work until you have gained substantial experience here at home.

Dr. Ralph W. Phillips, formerly with the Food and Agriculture Organization in Rome and now Director of International Organization Affairs, Foreign Agricultural Service in Washington, D.C., states:

"Opportunities in the international field are limited largely to posts which require men of fairly advanced training and experience. This usually means a minimum of a Master's or a Ph.D. degree, and perhaps ten or more years of professional and rather specialized experience. . . . If one wishes to aim at a career in the international agricultural field, the best way to prepare for it is by building a solid professional career in one's own country, so that he has a reputation to carry with him into the international area."

Dr. Hans Pederson, Chief of the Dairy Branch, Animal Production and Health Division of FAO in Rome, Italy, commenting on their Expanded Technical Assistance Program, states:

"To qualify for a position under this Program a candidate must have a degree in dairying and in addition some years of experience in dairy plant management or hygienic milk production or extension work, or a number of years' experience in dairy teaching at the university level."

Dr. J. C. Shaw, also an FAO executive, who is Chief of the Animal Production Branch in Rome, has this to say:

"The work of our own Headquarters staff and of our more temporary Field experts working under the Expanded Technical Assistance Program is primarily of an advisory nature to governments, and thus requires in most cases that such personnel have considerable training and experience prior to taking up such assignments."

Dr. Shaw, however, points out that there is nonetheless a dearth of qualified dairy personnel for short-term field assignments.

Dr. K. L. Turk, Head of the Department of Animal Husbandry, Cornell University, has written:

"There probably will be more opportunities in foreign service in agriculture for students with advanced degrees than for those with the B.S. degree. This is especially true for work with ICA, FAO, and various foundations."

In addition to the governmental, quasi-governmental, and foundation-type activities carried on overseas, there is a substantial inter-

national business community in the dairy industry. Several U.S. corporations have extensive international interests involving the manufacture and sale of dairy products overseas, as well as the export of U. S. dairy products. In general, the requirements of these companies for personnel would differ from those of the governmental-type organization, in that they frequently accomplish their own training. But they do recognize the need for technical training and they do require it.

Dr. Grover D. Turnbow is the Chairman of the Board of Foremost Dairies, Inc., and has in a number of special ways made great and lasting contributions to the foreign field of dairying. From San Francisco, California, Dr. Turnbow states:

"Business today requires men with technical training. They must know production, general operations, and salesmanship. In order to get ahead and become administrative executives they need experience in marketing. Never before have times changed so rapidly. . . . The old curricula are obsolete, although not quite as obsolete as a wagon or a buggy. Still, in order to keep up with the times there must be a very substantial change. . . . The opportunities in the foreign field are unlimited. A student should have or maintain a good grasp of the language of the country. Our company trains people before sending them abroad by giving them an intensive course in languages."

Dr. Turnbow concludes by pointing out that, in a foreign country, it is extremely important not to leave the impression of believing that just because you happen to come from the United States you belong to something unusual. The world is so small today that the international field is really a necessity.

Professor H. D. Kay, C.B.E., F.R.S., of Twyford Laboratories, Ltd., in England, has long given distinguished service to international dairying in many ways. Professor Kay's remarks are particularly pertinent. In part, they are as follows:

". . . the first essential is to have a thorough grasp either (a) of modern milk processing and milk products manufacture from the engineering standpoint, or (b) of dairy laboratory organization, methods, and application of laboratory findings, or (c) of methods of milk production, including fodder production, under temperate, semitropical, and tropical conditions."

"They must be self-reliant and able to work under climatic and other environmental conditions which may be very trying and, in relation to individuals and organizations, in some of the underdeveloped countries, with methods of thinking and acting which may be rather remote from Western methods."

Perhaps you have heard of the United Nations Children's Fund—(UNICEF). Mr. Donald R. Sabin, Coordinator of Food Conservation, is the key executive concerned with the worldwide dairy activities conducted by this organization.

Mr. Sabin has been extremely interested in helping with this paper. He recognizes the great need for qualified people in the dairy field, and sees many areas of activity in which to serve. But he also recognizes many problems in filling these needs. Mr. Sabin has stated:

"There is a big question, how much (some of) the countries will rely on American trained men when they themselves are training their own nationals. On the other hand, for several years to come the underdeveloped countries will have to rely to a fairly large extent on the service of foreign dairy-trained technicians to provide technical leadership to the industry."

Mr. Sabin emphasizes that the greatest need for the improvement of dairy practices lies in the tropical areas and the so-called devoloping countries with low per capita incomes. These would include India, Pakistan, South and Central America, the Middle East, and Africa. These countries, where the challenge is the greatest and which therefore require the best men, are lacking in resources to hire and maintain such men. Mr. Sabin points out, too, that in some countries the development of dairying is held back because it is increasingly difficult for the dairy cow to compete successfully with vegetable proteins.

Dr. James N. Warner serves dairying in the religious mission field and is head of the Department of Dairy Technology, Allahabad Agricultural Institute, Allahabad, India. Dr. Warner has indicated his belief that the post to which he is presently assigned is "the only position in the whole mission field where highly specialized training in dairy technology is fully used."

He points out, however, that there are many places in the mission field where a person can use dairy training, but the duties are primarily in the area of general agriculture, including both dairy and animal husbandry. Dr. Warner indicates that the Institute during the month of April was calling for a dairy engineer to join its Department of Agricultural Engineering, to help with an expanded program of training dairy specialists at the Bachelor of Science level under Allahabad University. This man would be required to have at least an M.S. degree. His duties would involve teaching animal husbandry and dairying. For anybody interested, I have the name of the person to contact for further details. Dr. Warner has furnished us with a number of other calls for agriculturalists in the mission field, including the following locations: Belgian Congo, Korea, Guatemala, Pakistan, and India. I have Dr. Warner's notes on these and will be happy to refer you to them if you are interested.

Dr. C. P. McMeekan, Director of the Ruakura Animal Research Station, Hamilton, New Zealand, sends us a very keen and practical observation:

". . . it seems to me that the opportunity of making a worthwhile career (in an economic sense) in the dairy industry in the international and foreign field is strictly limited. In my view this is so because of the vast difference in the salary levels paid in all fields of science and technology in countries outside the United States, relative to those paid within it.

"For example, I have had several United States students studying with me here to whom I was prepared to offer a very worthwhile career in the field of dairy cattle husbandry in New Zealand. While our salary levels are in keeping with local living costs they are, in absolute terms, only a fraction of those prevailing in the United States. This unpalatable fact resulted, in each case, in the young men concerned returning to their own country. This situation perhaps highlights the main points concerned. Any young man intending to enter the international sphere has to be prepared to accept on a permanent basis the economic conditions prevailing in the foreign country or countries concerned."

When President Meyer of the National Student Branch invited me to participate in this symposium he suggested a straight-from-the-shoulder, no-holds-barred picture. I hope this has been it.

Someone once said: "To achieve success a young man must find a Cause bigger than himself to diligently serve and love, and he must diligently serve and love this Cause throughout his lifetime."

In considering the international side of selecting a career in the dairy industry, it would appear to be prudent to underline the foregoing quotation, even though its application may be as pertinent to a domestic career. These remarks, however, have pointed up what I consider to be relatively somber, near-term prospects, if we are to "bar no holds." As Dr. McMeekan has concluded specifically with examples, and others have implied, the best financial prospects for a dairy career are right here at home and not in the international field, at least for the relatively inexperienced graduate, and at this time.

The largest reservoir of position demand in the foreign field of dairying would appear to be in the governmental and international agency field, such as: University programs in foreign countries, the ICA technical assistance program; FAO; UNICEF; FAS; and others. Unfortunately, for the new and relatively inexperienced graduate, the qualifications mostly require advanced degrees as well as a number of years' experience. An exception to this rule would be employment with one of the many reliable and substantial commercial companies doing business on an international basis. These companies, however, generally have their own training programs.

But we are far from being justified in completely discouraging a career in the field of international dairying. On the contrary, our information has established beyond any question of a doubt the over-riding importance of this area of endeavor and the fact that inescapably you will be spending more time in the foreign field.

I believe if I were to state a single most important conclusion of this talk it would be this: Keep your eye on the international field, but first establish yourself in a career right here at home.

Remember, "No great thing is created suddenly." But I would submit that the foreign field of the dairy industry needs good men badly, and that in this phase of our industry you can, in time, find "a Cause diligently to serve and love throughout a lifetime."

OUR INDUSTRY TODAY

PROBLEMS OF MILK SUPPLY ASSOCIATED WITH POSSIBLE NUCLEAR REACTOR ACCIDENTS

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The increasing number of power reactors and other proposed nuclear energy uses carries with it the threat of accidental radioactive contamination of the local food supply. It is probably safe to say that as the number of installations increases, the probability of accidents also increases. The Windscale incident (1) in England demonstrated that such accidents can happen and can create troublesome contamination problems. In the event of such an accident in this country, one of the first actions should be to restrict the milk supply to nonhuman uses. Milk would probably be the food product of primary or sole concern, as was the case at Windscale. This would depend to some extent on the crops grown in the area and the season of the year. However, this discussion will be concerned only with the problem of milk supply. Much attention has been given in this country to procedures to be followed in the event of nuclear disasters, but little attention has been given to milk supplies in the case of reactor accidents.

The problems discussed here are related to handling the situation of a significantly contaminated milk supply. The questions raised are certainly secondary to monitoring and protection of public health by restricting the milk supply. The problems discussed are primarily problems not of public health but of public relations. However, the management of an accident of this type is likely to be evaluated more on the grounds of public relations than on a public health basis. An accident of the type at Windscale, followed by indecision and vacillating directives, could do irreparable harm to the nuclear energy program in the United States. An attempt will be made to show that broad policies can and should be made in advance, which would eliminate the need for hurried and possibly ill-considered decisions in the heat of an emergency.

EXTENT OF THE PROBLEM

Some idea of the magnitude of a nuclear reactor accident can be obtained from the Windscale experience. An area of approximately 30 mi. along the coast and 6-10 mi. inland (about 200 mi.^{2}) was under restriction for 2-5 wk.

In Table 1 is presented a list of current and proposed civilian reactor establishments, together with a crude estimate of the number of dairy cattle in the vicinity. This estimate is arrived at by considering those counties which have a majority of their area within a 30-mi. radius of the installation. Counties are the smallest subdivision for which dairy cow numbers are readily available. The areas included and, consequently, the number of cows, may be considered too large. However, the Windscale accident is the only large-scale incident for comparison, and it should be pointed out that the following several factors operated to keep their problem below the maximum of severity: (a) Weather conditions were about normal; (b) the restricted area was bordered on its long side by the Irish Sea; (c) the accident occurred in October, when a change to stored feed could be made easily; and (d) the only significant radionuclide released was I^{131}, which has a short physical half-life (eight days). The point is, that the situation could be much worse.

A conservative estimate is that a cow will produce 20 lb. of milk per day. The per capita consumption of fluid milk in the United States is about 1 lb. per day. Thus, each cow represents the milk supply for about 20 people, and if 10,000 cows are involved, potentially the milk supply for 200,000 people could be affected. The milk production of 10,000 cows would have a farm value of $6,000 to $12,000 per day. The Rowe, Massachusetts, area (see Table 1), for example, received an average of about $40,000 per day from milk sales in 1959.

HANDLING OF CONTAMINATED MILK

The first question to be answered in preparation for such an emergency is the importance of fluid milk production in the area. Would restrictions result in a significant decrease in the fluid milk supply for some metropolitan area? If milk production is primarily surplus fluid milk, or milk produced for manufactured dairy products, the problem will be considerably simplified. The areas listed in Table 1 primarily would be producing milk for the fluid market.

The season of year, obviously, is going to influence the course of action, at least in the northern states. In the Northeast and Midwest, cows are stabled throughout the winter. Farther south and in western states, cows commonly are allowed access to fields or pasture, although

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TABLE 1

Location of civilian reactors[a] (2)

Reactor name and location	Output MW (e)[b]	Date of criticality	No. dairy cows (nearby counties)	States included
1. Vallecitos, California	5	8-5-57	10,000	California
2. Shippingport, Pennsylvania	60-90	12-2-57	80,000	Pennsylvania, Ohio, West Virginia
3. Dresden, Illinois	180	10-15-59	30,000	Illinois
4. Rowe, Massachusetts	134	October, 1960	35,000	Massachusetts, Vermont, New Hampshire, New York
5. Elk River, Minnesota	22½	November, 1960	80,000	Minnesota
6. Lagoona Beach, Michigan	100	End 1960	9,000	Michigan, Ohio, Ontario
7. Indian Point, New York	275	April, 1961	40,000	New York, New Jersey, Connecticut, Pennsylvania
8. Piqua, Ohio	12½	July, 1961	60,000	Ohio
9. Sioux Falls, South Dakota	66	1962	50,000	South Dakota, Minnesota, Iowa
10. Hallam, Nebraska	75	1962	50,000	Nebraska, Kansas
11. Big Rock Point, Michigan	50	1962	.....	.....
12. Saxton, Pennsylvania	5	February, 1962	60,000	Pennsylvania
13. Parr Shoals, South Carolina	17	June, 1962	15,000	South Carolina
14. Humboldt Bay, California	70	Mid-1962	25,000	California
15. Peach Bottom, Pennsylvania	30-40	December, 1963	150,000	Pennsylvania, Maryland, Delaware

[a] Reactors built by the AEC or national laboratories are not included in this table.
[b] Megawatts (electrical).

the amount of grazing is uncertain. In this case, it may or may not be necessary to shut up these cows. In southern states, cows are likely to be on pasture at any time. During the normal pasture season, it may not be necessary to restriet all herds. Some herds are kept in lots and fed dry feed the year around. In some regions, this is a common practice, although there are few places where it is universal.

Rather than dumping milk, will it be sufficient to restriet cows to a lot and feed them hay and grain produced prior to the accident? This raises the question of whether imported hay from uncontaminated areas will be subsidized in some way to support these herds.

Another question has to do with disposal of milk. A commercial dairy farm can not use several hundred gallons of milk per day for animal feed. Milk can be fed to cows, but it would seem that this practice should be prohibited. Fat, the highest-priced fraction of milk, is commonly essentially free of radioactivity. To a lesser extent, this applies to cream. Will such products be allowed for sale? If the major contaminant is I^{131}, it would be possible to turn this milk into manufactured dairy products and allow sufficient storage time for decay to remove the radioactivity. It is easy to imagine sales resistance to these products. Would it be worth while to dry the milk for animal feed? Still another possibility is to mix contaminated milk with a large volume of uncontaminated milk. Should this possibility be considered, or rejected on principle?

Another question likely to be raised is the use of equipment to remove radionuclides. Such methods are available, and a pilot plant to carry out this operation is reported to be under construction.

It is quite possible that none of these alternatives would be considered under present conditions, when the federal government is purchasing surplus dairy products. A policy could be established nationwide on this matter, which would obviate the need for discussion of most of the points covered in the section above.

EMERGENCY MILK SUPPLY

An alternative supply of milk for the market is probably more a psychological than a nutritive necessity. There may be serious problems in getting an emergency supply of fluid milk, which will depend to some extent on the season of the year. Early fall is a period of short supply in most areas. An alternate supply may be available 50 mi. away, or it may be necessary to go 1,000 mi. If long hauls are necessary, will subsidies be made available? Will each handler be responsible for obtaining a supply to satisfy his customers, or will responsibility for obtaining a supply be turned over to a producers' cooperative—if such exists? These are the organizations which assemble milk for many metropolitan areas, but not all.

Plans would also have to be made to supply milk to rural areas where deliveries are not normally made. Also, a decision needs to be made on disposition of milk which distributors still have on hand, that was produced after contamination occurred. If several days have elapsed before a decision is made as to restriction (which should not be the case), should the public be told to dispose of milk supplies they have at home? This is not a simple question because, under present methods of distribution, milk delivered to the home may be two to seven days old.

COMPENSATION AND INSURANCE

Civilian reactors in this country have liability insurance issued by organizations formed of groups of insurance companies. Presumably, compensation would be made to producers for lost milk sales. Milk pricing is universally recognized as the most complex of any farm product. Some attention to this subject in advance might save ill will and litigation. Milk prices range from $2.20 to $7 per hundredweight. Within the same market, ranges from $3 to $6 are common. Any plan of compensation should be devised with the help of competent advice, because of the complexities in indemnification certain to arise.

A record of production during the period of restriction would also seem to be a necessity for use by the compensating agency. There is a dairy organization (Dairy Herd Improvement Association) which normally handles milk production records and probably could provide the same service for the agency providing compensation for lost milk.

PUBLIC RELATIONS

Another matter which seems worthy of consideration is the publicity or educational program which will be necessary. Can it be decided who will speak on the problem with the voice of authority? Someone must speak and make decisions which affect the public on one side and producers on the other. Will the person who speaks have the support of the other responsible agencies? There will be enough diversity of opinion expressed without public agencies presenting uncoordinated or conflicting statements.

Attention might well be given to preparation of authoritative information which could be used in event of an emergency. Such information would discuss in laymen's language such matters as the derivation of maximum permissible concentrations, the mechanisms of incorporation of radionuclides into the food chain, and monitoring methods which will be used. To get the problem on the basis of science and out of the realm of mysticism will require real talent in the educational program. A program of this type can and should be prepared at the national level.

WHAT AGENCY WILL BE RESPONSIBLE FOR HANDLING THE EMERGENCY?

If it is agreed that fluid milk will be eliminated from the market, the question then is, who or what agency is going to make the decision, and who is to enforce the directive? This would seem to be the role of a public health department. However, there is no consistent organization of such departments throughout the country. City or county health departments are concerned with the milk supply sold in their area of jurisdiction and not necessarily with the milk produced in their area. It is not uncommon for neighboring farms or even the same farm to be regulated by two or more health agencies from distant cities located in two or more states.

The problem will be to stop the sale of milk produced in the restricted area, and this can not be done in most cases by merely shutting off the local milk supply. Public health agencies generally have no direct supervision over or contact with manufacturing plants or their producers. State health departments may or may not be equipped to deal speedily with such an emergency. Furthermore, it is quite possible that two or more states would be involved, as indicated in Table 1. A group outside the control of any agency are the farms which have one or two cows for their own milk supply. This group is potentially in the gravest danger from contaminated milk.

If possible, it would seem desirable to be able to notify producers without resort to public media. This would require that someone know who the producers are. This suggestion does not mean that the public will not be informed about the situation.

There is no shortage of agencies that would be involved in such an emergency, but which would be given authority to deal with the problem should be determined in advance. Some areas have federal marketing orders, which have a close contact with fluid milk producers. Producers' cooperatives have even closer contact, but no regulatory powers. This problem might be considered within the province of the Food and Drug Administration, but this organization would not ordinarily have the field force on the scene to deal with such a problem. The Agricultural Extension Service has the field force and contacts, but is an educational and not an enforcement organization. State departments of agriculture vary from state to state in the degree of control over the dairy business. There are generally divisions of authority and overlapping of authority between these departments and public health departments.

What role would Civil Defense organizations play? United States Department of Agriculture agencies have been organized on a county basis to deal with food supplies in the event of nuclear bombings. Would the problems dis-

cussed here come within their province? If police are to be brought in to ensure enforcement, what branch of police? And who is to tell them what to enforce? To summarize, who is to restrict milk sales, and who will enforce the restriction?

A study of reports would indicate that the problems presented here were handled speedily and efficiently following the Windscale accident. This is no reason to believe, however, that the same would necessarily be the case in this country. The organization of agencies which might be involved is more localized in this country and it is felt not as susceptible to central organization and coordination within a short period of time. The United Kingdom is a smaller country and better suited to national mobilization of the necessary staff.

CONCLUSIONS

The above discussion perhaps leaves one with the impression that a great many questions have been raised but that no solutions have been offered. In theory, at least, the solution is simple. What is needed is a group to take the responsibility of planning for a course of action in event of a reactor accident. As pointed out, none of the existing agencies is specifically equipped to deal with the problem. It is hoped that the need for planning has been demonstrated. Planning will essentially involve coordinating the ideas and activities of the many agencies involved and which have information or services necessary to the success of the program. Some of the necessary decisions can be made at the national level for nationwide application, but many decisions must be made on the basis of local conditions. Local conditions, however, will embrace several counties and perhaps more than one state.

The dairy industry should consider taking the leadership in drafting a plan of action which will protect their business, since it is obviously impossible to assign the problem to any existing agency. Certainly, no group has more to gain than the dairy industry from a well-conceived program for the protection of the public milk supply.

Accidents of the type envisioned here may be a remote possibility, but no one will argue that they are impossible. The time and cost involved to prepare for the exigencies described would be small compared to the over-all cost involved in assuring the maximum of safety for reactor establishments.

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FACTORS AFFECTING CHLORIDE CONTENT AND RELATIONSHIP OF CHLORIDE, PROTEIN, AND SOLIDS-NOT-FAT IN GUERNSEY MILK

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Various investigators (4, 6, 7, 11) have recommended the use of chloride content of milk to increase the accuracy for lactometric determination of solids-not-fat. Although chlorides comprise only about 1% of the solids-not-fat and lactose more than 55%, it may be assumed from the reported research that the accuracy of the solids-not-fat determination could be improved by taking chloride content into consideration. The ease with which the chloride content of milk could be determined is a decided advantage in favor of the suggestion. In contrast, the determination of lactose involves the precipitation of protein, which introduces the risk of errors in the analysis. These errors might be attributed to some lactose bound in the proteins.

A possible approach to the accurate lactometric determination of solids-not-fat content of milk is to use the information on chloride and total protein content to allow for any effect they may have on lactometer reading for solids-not-fat. Total protein can be determined by rapid dye-binding methods, using Orange G or Buffalo Black dyes, which produce nearly identical results with the standard Kjeldahl method. If this approach is to be used, it is necessary to obtain information on the factors affecting chlorides and the relationship of total protein, chloride content, and solids-not-fat by lactometric determination.

MATERIALS AND METHODS

Milk samples from Guernsey cows on Cornell's McDonald Farms near Cortland, New

York, were used. A total of 725 samples from 133 cows was analyzed for chloride, milk fat, total protein, and solids-not-fat during a 10-mo. period from August, 1959, to May, 1960. The milking cows at McDonald Farms are kept under optimum conditions of feeding and management, standardized as much as possible for maximum results. The same grain mixture has been fed at a practical feeding rate over a period of several years to help provide standardized conditions. The effect of mastitis on chloride content of the milk could not be studied because too few cows showed clinical symptoms of mastitis. This may be an advantage for reliability of results concerning the factors studied.

Chloride. The potentiometric method of determining chloride, suggested by Herrington and Kleyn (10), was followed in analyzing milk from individual cows. Aluminum potassium sulfate was used to minimize the interfering effects of proteins in milk. Samples were not analyzed in duplicate after sufficient skill was acquired. It was an easy matter to conduct the test, because both the sample and the silver nitrate solution used in the titration were mixed by a magnetic stirrer. Reading the millivolts was equally easy.

Total protein. This was analyzed by the Kjeldahl method.

Milk fat. Duplicate Babcock tests were made on each sample.

Solids-not-fat. Lactometer readings were taken with a 6-in. Watson-type lactometer (WEDCO) at 102/102 ± 1.0° F. The lactometers used were calibrated by the USDA Research Laboratory at Beltsville, Maryland. The solids-not-fat content of the milk was then calculated by using the formula of Whittier (19), with the modification suggested by Watson (18).

RESULTS AND DISCUSSION

Effects of stage of lactation and age on the chloride content. A summary of the data on percentage of chloride as affected by the stage of lactation and age is shown in Table 1. Chloride content increased as lactation and age advanced. When first and second, third and fourth, and fifth to eleventh lactations were combined, the weighted means were 0.079 ± 0.0004, 0.102 ± 0.0016, and 0.116 ± 0.0020, respectively. The changes, with a trend upward from the first to the tenth month of lactation in each group, respectively, were: 0.074 to 0.085, 0.080 to 0.128, and 0.096 to 0.136. These observations agree with published research (6, 12, 14, 16, 17). The values in this research are lower than those previously reported in references mentioned. This might be due to the differences in the methods of analysis or to the very low incidence of mastitis in this particular herd.

Of the milk constituents analyzed, chloride showed the highest coefficient of variation of 18.5 ± 2.1%, as influenced by stage of lactation with all ages combined. This confirms the work of Reder (16), who observed coefficients of variation from 9.4 to 22.1% for changes during lactation in Jersey milk.

There was a sizable increase of chloride even during the second to the sixth month of lactation in all ages. This may indicate that the increase in chloride is not influenced by gestation to the extent observed for solids-not-fat. Possibly, the increase is due to decreased milk production as the lactation period progresses. Sharp and Struble (17) reported a marked increase in chloride when the production of Jersey and Guernsey cows fell below 15 lb. per day. On the other hand, Krienke (11) reported that for several cows there was no change, or only a slight increase, in chloride content at the end of the lactation period.

The increase of chloride as the cows become older is in contrast to a decrease for solids-not-fat and protein. Ragab *et al.* (15) reported that buffaloes with four or more lactations had the highest chloride content.

TABLE 1

Effect of stage of lactation and age on percentage of chloride

Month of lactation	No. of samples [a]	First and second lactations	Third and fourth lactations	Fifth to eleventh lactations
		360 samples	181 samples	184 samples
1	66	0.074 ± 0.0018	0.080 ± 0.0019	0.096 ± 0.0040
2	85	0.074 ± 0.0015	0.088 ± 0.0030	0.104 ± 0.0042
3	88	0.078 ± 0.0019	0.089 ± 0.0018	0.116 ± 0.0070
4	88	0.078 ± 0.0019	0.097 ± 0.0012	0.113 ± 0.0068
5	82	0.081 ± 0.0019	0.099 ± 0.0035	0.116 ± 0.0054
6	76	0.082 ± 0.0018	0.112 ± 0.0030	0.122 ± 0.0069
7	66	0.080 ± 0.0020	0.109 ± 0.0048	0.120 ± 0.0066
8	64	0.081 ± 0.0018	0.117 ± 0.0019	0.121 ± 0.0052
9	58	0.081 ± 0.0025	0.126 ± 0.0083	0.124 ± 0.0052
10	52	0.085 ± 0.0030	0.128 ± 0.0102	0.136 ± 0.0071
Weighted mean		0.079 ± 0.0004	0.102 ± 0.0016	0.116 ± 0.0020

[a] Number of samples in terms of 725 cow months for 133 cows.

If the negative relationship of chloride and lactose has to be maintained by the cow in order that the osmotic pressure of the milk is not disturbed, then a high chloride content is associated with low lactose percentage, and vice versa. Since lactose and chloride account for 80% of the osmotic pressure, there tends to be a constant relationship between the two (4, 7). Further, since chloride content increased as cows became older, it may be assumed that lactose decreased correspondingly. This assumption contradicts the findings reported by Leydolph and Ulrich (13), that lactose content of Holstein-Friesian milk increased from 4.57 in the first lactation to 4.85% in the fourth, and also D'Alo's (5) contention that lactose is not affected by the age of the cow.

Partial correlations of chloride with lactometer reading, solids-not-fat, and fat. There was a highly significant zero-order correlation of −0.722 between chloride and lactometer reading. Holding casein or total protein or fat statistically constant did not significantly affect the first-order correlations. However, when fat and total protein were held constant, the second-order correlation between chloride and lactometer reading increased significantly to −0.839. This indicates that fat and total protein depressed the relationship. There was a highly significant zero-order correlation between chloride and solids-not-fat of −0.733. When total protein was held constant, the first-order correlation increased to −0.910. This is expected, because when total protein is removed from solids-not-fat, the other major constituent, lactose, has a greater influence. The first-order correlation of −0.910 might be assumed to be the zero-order correlation of chloride and lactose. This assumption agrees with the observations reported on the relationship of chloride and lactose by Barry and Rowland (1) and Chanda *et al.* (3).

The zero-order correlation of −.073 between chloride and fat was not significant. However, holding total protein constant gave a highly significant first-order correlation of −0.260. Likewise, holding whey protein constant increased the relationship to −.270, which was highly significant.

Effect of stage of lactation on the relationship of chloride, total protein, and solids-not-fat. The weighted means of all the tests for all cows in each stage of lactation, regardless of age, were computed. The results are presented in Table 2. Solids-not-fat content was lowest in the second month, which is coincident with the highest milk production and low fat test. Total protein content also was low during this month, in contrast to chloride content. The latter started on the upward trend, which extended throughout the lactation. In all probability, the increase in chloride content was accompanied by a decrease in lactose, although probably not entirely to a proportionate degree.

TABLE 2

Effect of stage of lactation on percentage of total protein, chloride, and solids-not-fat

Month of lactation	No. of samples [a]	Solids-not-fat	Total protein	Chloride
		(%)		
1	66	9.15	3.40	0.081
2	85	9.04	3.39	0.085
3	88	9.17	3.47	0.091
4	88	9.18	3.64	0.092
5	82	9.27	3.79	0.095
6	76	9.29	3.91	0.099
7	66	9.33	3.97	0.097
8	64	9.31	4.06	0.098
9	58	9.27	4.09	0.103
10	52	9.23	4.08	0.110
Average—weighted according to milk production		9.21	3.69	0.093

[a] Number of samples in terms of cow months—a total of 725 samples from 133 cows.

If lactose falls with the rise in chlorides in milk to maintain proper osmotic pressure, it is possible that the rise in total protein is largely responsible for the increase in solids-not-fat. It is likewise possible that the decrease in lactose may not be entirely proportionate to the increase in protein and, therefore, exert an influence on solids-not-fat content.

The subsequent increase or decrease of solids-not-fat as lactation advances could be attributed to the sum of the effects of protein and lactose, since chloride is only a minor constituent of solids-not-fat. Krienke (11) suggested that an increase of 0.01% chloride is reflected by a decrease of 0.17% lactose; thus resulting in a decrease of 0.16% solids-not-fat. The highly significant negative correlation of chloride and solids-not-fat (− 0.733) partially corroborates Krienke's assumption. This is so because of the presence of total protein, which accounts for about 40% of solids-not-fat. Furthermore, total protein and chloride are positively correlated with $r = +0.280$, which is highly significant. The influence of protein results in an over-all increase in solids-not-fat at the time the chloride increases. This is shown by the following multiple linear regression equation of:

$\hat{Y} = 8.33 + 0.3282\ X_1 - 3.5462\ X_2$, where $\hat{Y}$ = predicted solids-not-fat; X_1 = percentage total protein; and X_2 = percentage chloride. The standard partial regression coefficients of: $b'_{Y1.2} = 1.248$ for total protein and $b'_{Y2.1} = 0.384$ for chloride show that total protein is relatively more important than chloride in determining solids-not-fat as affected by the stage of lactation. This may suggest that there is a need for a fast and accurate method of analyzing lactose, so that it could be used in addition to total protein in determining solids-not-fat.

Effect of season on the relationship of chloride, total protein, and solids-not-fat. Table 3 shows the interrelationship of chloride and

TABLE 3

Effect of season on the relationship of chloride, total protein, and solids-not-fat from second- to the sixth-month stage of lactation tests

Month	No. cow months [a]	Solids-not-fat	Total protein	Chloride
		(%)		
August	41	8.93	3.48	0.092
September	51	9.16	3.64	0.093
October	50	9.34	3.89	0.096
November	46	9.28	3.70	0.094
December	43	9.34	3.69	0.093
January	27	9.27	3.60	0.089
February	38	9.21	3.60	0.087
March	41	9.25	3.57	0.089
April	42	9.22	3.47	0.091
May	40	9.09	3.48	0.096

[a] A total of 419 samples from 115 cows.

total protein content with solids-not-fat during different months of the year. The tests from the second to the sixth months were used to minimize the effect of decreasing milk production and gestation. There was a rise in the solids-not-fat from a low of 8.93% in August to a high of 9.34% in October and December. These increases might be attributed to the increases in total protein, which are more than enough to cover up the decreases in lactose due to the slightly higher chloride content. When January is compared with May, the chlorides show an increase which should be accompanied by a decrease for lactose. Since total protein showed a decrease, the over-all effect is for solids-not-fat to decrease.

There was a considerable variation in the chloride percentage as influenced by months of the year. The coefficient of variation of $25.2 \pm 2.2\%$ for months is higher than that observed for stage of lactation. Based on research reports in the literature, it may be expected that the high variability of chlorides was accompanied by a high variability of lactose. This is in line with the findings of Davies (7) and Brown *et al.* (2), who reported marked variations in lactose. However, Eckles and Shaw (8) reported that lactose is the least variable of the major constituents of milk, since 90% of samples of a given lactation showed a variation of less than 0.2% lactose. Filipovitch and Filipovitch (9) report that high chloride was not found to be related to the physical and chemical properties of milk; in their study they obtained high chloride values from both undernourished and well-fed cows under good management.

The relationship of the three constituents as influenced by seasons is shown in the following multiple linear regression equation of:

$\hat{Y} = 7.55 + 0.7867\ X_1 - 12.9001\ X_2$, where $\hat{Y}$ = predicted solids-not-fat; X_1 = percentage total protein; X_2 = percentage chloride. The standard partial regression coefficients of: $b'_{Y1.2} = 0.806$ for total protein and $b'_{Y2.1} = 0.310$ for chloride show that the per cent of total protein is relatively better than chloride content as a possibility to use for improving the accuracy of solids-not-fat tests by giving consideration to the effect from variations in milk constituents.

SUMMARY

A total of 725 milk samples from 133 Guernsey cows was analyzed for their content of chloride, total protein, milk fat, and solids-not-fat. The chloride content increased as lactation and age advanced. Weighted means for chloride content were 0.079 ± 0.0004 for first and second lactations combined, 0.102 ± 0.0016 for third and fourth lactations, and 0.116 ± 0.0020 for fifth to eleventh lactations. The changes, with a trend upward in chloride content, from the first to the tenth month of lactation in each group, respectively, were 0.074 to 0.085, 0.080 to 0.128, and 0.096 to 0.136%. The protein and solids-not-fat content also increased as the lactation period progressed. Seasonal trends were more evident in total protein and solids-not-fat percentage than in chloride content. Total protein was relatively more important than chloride content as a possibility to use for improving the accuracy of lactometric determination for solids-not-fat.

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ASSOCIATION AFFAIRS

ANNUAL REPORT OF THE JOURNAL MANAGEMENT COMMITTEE (1960-1961)

F. J. Doan, Chairman
Department of Dairy Science, Pennsylvania State University, University Park

Plans for handling the interpretive summaries after January 1, 1961, were agreed upon. Members of the committee and the Editor will assist the Secretary with the editing and he will arrange and forward them to the Garrard Press for publication in the quarterly authorized by the Executive Board at the June, 1960, meeting.

The name A.D.S.A. Review was decided upon for the quarterly, as was also a self-cover containing the lamp design now used on copies of the constitution and on the descriptive folder employed by the Membership Committee.

Proposal No. 2 of the Garrard Press was accepted for printing and mailing the A.D.S.A. Review. This calls for printing by letterpress, self-cover, double column, 9 pt. black type, etc., and second class mailing. The cost of this, according to Garrard Press figures, will be no greater than the present cost of printing the summaries in the Journal.

Summaries will have a new heading in the quarterly, but the full title and the author(s) affiliation will be at the bottom of the text.

Publication dates were set for April, July, October, and January, as early in the respective months as possible. The first issue appeared in April, 1961. Copies are being mailed to all Association members and to a selected list of publications.

A statement concerning the interpretive summaries was prepared and published in the October Journal. This reviewed the background, the objects in view, and requested authors to prepare the summaries carefully, keeping the desired goal in mind. Reprints of the statement were obtained by the Editor for sending to authors of papers accepted for publication.

It is realized that some summaries submitted are not as satisfactory as might be desired; some papers do not lend themselves well to popularization; the time factor may interfere with editing in some cases; and some summaries will doubtless have to be used as received. Nevertheless, it was deemed desirable to include summaries for all papers published in the Journal insofar as this is physically possible. On the other hand, many summaries are quite satisfactory as written and it is hoped that, with time, authors will become increasingly proficient in preparing them to meet the objective of the program.

It is the understanding of the Journal Management Committee that a survey of membership opinion is to be made after four issues of the A.D.S.A. Review have been mailed, but not later than when six issues have been received by the membership. On the basis of this opinion survey, and the financial considerations involved, the Executive Board will then decide whether the quarterly is to be continued, discontinued, or modified to a more comprehensive publication.

It was reiterated that A.D.S.A. members may publish research papers on subjects pertaining to economics, marketing, etc., provided they meet the standards of quality.

The majority of the Committee feels that good papers in the areas of economics and marketing would improve the Journal's usefulness and appeal to a large segment of the membership. Papers by nonmembers may, on occasion, be published in the Our Industry Today section if they are of sufficient general interest and importance, and provided they are organized and well written. At present the Editorial Board has no representative in these subject matter areas, but there are competent and, doubtless willing, Association members who might be called upon for this service.

In considering the financial status of the Journal, it appears likely that the operation for 1961 will end in a deficit. The following points are emphasized:

(a) Journal size is a determining factor in costs. It has increased in the last few issues. The 1961 budget was based on an average size of 195 pages (180 without the interpretive summaries). If the average size is much above this, the expected loss may increase considerably. Each additional page increases yearly printing costs by $300. The January, February, and March issues this year averaged 22 pages over the 180 estimated. This adds $6,600 to the yearly cost.

(b) If it is impossible or undesirable to hold the size of the Journal to the suggested number of pages, an increase in members' dues may be needed to balance the budget.[1] Present dues are low compared with comparable organizations.

(c) Costs of printing and mailing the Journal for the last few issues have amounted to $1 per copy. For 12 issues this would be $12, or $2 more than the regular membership dues and $7 more than affiliate dues.

[1] Dues were increased by action of the Executive Board. (J. Dairy Sci., 44: 1580. 1961).

The detailed budget figures highlight the importance of advertising as a source of revenue. The Secretary-Treasurer has been putting a great deal of time and effort into procuring advertising. He feels that if the members were more aware of the role of advertising in permitting annual dues at the present low rate, he might be able to obtain more assistance from members in making advertising contacts and maintaining present ones.

The financial status of the Germ Cell Damage supplement, published last year in the JOURNAL, indicates that we will do no more than break even on this venture.

The Committee was alerted to the considerable increase in the number of nonpaying members of the Association in the last few years. This number now totals 57, made up as follows:

Charter members	2
Honorary members	13
Life members	42

As of January 30, 1961, there were 199 who will have been members of the Association for 25 years or more and who are, therefore, eligible for Life Membership as they retire. It is felt that this gratuity may reach disproportionate totals.

As was indicated in last year's report, the problem faced by the Journal Management Committee and the Executive Board is to determine what the future needs of the Association are with respect to publications (granting that no radical change is to be made in the type of membership).

1. Shall the JOURNAL and the A.D.S.A. Review be continued about as they are now?
2. Shall the Review be discontinued and the JOURNAL continued on the present basis?
3. Shall the Review be made more comprehensive by adding some material now in the JOURNAL and some other features?
4. Shall all material other than research papers and technical notes be removed from the JOURNAL and put in the Review?
5. How shall two publications be financed if it develops that this seems the logical move?
6. What other possibilities should be considered?

In connection with these questions, the Executive Board, at the June, 1961, meeting, authorized the Committee to make a study of the possibilities under No. 3 and 4, taking into account the financial limitations, and to prepare a report to the Executive Board at a possible mid-year meeting. It was also suggested that a membership questionnaire be formulated, on the basis of the findings, to be submitted as a part of the questionnaire previously authorized in connection with the future of the Interpretive Summaries.

Respectfully submitted by the Journal Management Committee:

J. C. CAVANAUGH
H. A. HERMAN
E. O. HERREID
H. F. JUDKINS
F. J. DOAN, Chairman

CALL FOR PAPERS FOR THE 1962 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

M. E. SENGER, Chairman, Program Committee, A.D.S.A.
Department of Animal Industry, North Carolina State College, Raleigh

The 57th Annual Meeting of the American Dairy Science Association will be held June 18-21 at the University of Maryland, College Park. Members who wish to present papers must submit titles and abstracts not later than March 1. This deadline must be met to permit publication of titles with the complete program in the April, and abstracts in the June, JOURNAL.

All members of the Association, including graduate student affiliates, are entitled to present papers. Participation by members of the industry and by senior members of the Association is especially encouraged. The Program Committee favors the general policy that an individual present only one paper and that his name appear as author on no more than two. This Committee, together with the Association membership, wishes to stimulate vigorous, sound research and realizes that these restrictions may penalize some members engaged in full-time research. Therefore, the Committee has liberalized this policy so that a member may exceed these limits, but only if he or his department rates the abstracts in order of preference for oral presentation.

Papers submitted for the annual meeting should be confined to research that has not been reported. Abstracts of papers accepted for publication by a scientific journal before the annual meeting are not eligible, as this constitutes duplication. If the total number of papers submitted by the membership is too great to include in the program, the Committee will assign some papers to be read by title only. In this event, consideration will be given to quality of research to be reported, number of abstracts per author and department, and im-

portance of the research. Abstracts arriving late will be rejected.

Attention is called to the Dairy Manufacturing Extension Section program and members are urged to participate. This is a subsection of the Manufacturing Section.

Mimeographed copies of pertinent data are desirable for distribution when the paper is presented. At least 250 copies should be made available. This can be supplemented by slides for projection on screens, provided the author can adhere to the assigned time of 12 to 14 minutes for presentation of each paper.

PREPARING ABSTRACTS

The Program Committee encourages continued improvement in quality of papers and in oral presentation. Careful design of experiments and proper interpretation of results are necessary. Strict compliance with the instructions for preparation of abstracts will simplify the task of the Program Committee, and will improve the program. *Careful editing of abstracts before submission is essential.* Each year a number of abstracts must be returned due to incomplete details. Please follow instructions for preparing abstracts carefully.

1. All abstracts must be submitted on regular 8½- by 11-inch paper.
2. Abstracts must not exceed 200 words by actual count. Those exceeding 200 words will be returned to the author for revision.
3. An original (on bond paper) and three copies of each abstract should be typed double-spaced. The original and one copy should be mailed to the Chairman, another copy to the Vice-Chairman, and the fourth to the Secretary of the Section where the paper will be presented. The original copy will be used for publication in the JOURNAL.
4. The style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the June, 1961, JOURNAL for guidance.
5. Only initials of authors should be used, except in unusual cases where it may be necessary to use the complete name.
6. When more than one author is listed, indicate who will present the paper by an asterisk after his name.
7. The title should indicate clearly the nature of the research. It should not be repeated again in the text. The abstract should include, insofar as possible, the design and major results of the investigation. Only complete research should be reported. Brief, essential statistics will make the data more meaningful.
8. The following form with no caps for the title is correct: Utilization of carbohydrates posterior to the rumen-reticulum of the bovine. J. T. Huber and N. L. Jacobson, Iowa State University.
9. If the author lists an address for an experiment station other than the University, such as a USDA research branch or a commercial company, the complete address should be provided, as in the following example: A study of dye reduction methods as platform tests for the detection of antibiotics. Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.
10. All symposium papers should be typed double-spaced and organized according to the style used in the JOURNAL OF DAIRY SCIENCE. The author should send the first copy direct to the JOURNAL Editor, E. O. Herreid, before or immediately following the Annual Meeting.

GRADUATE STUDENT PRESENTATION CONTEST

This contest will be conducted in both the Production and Manufacturing Sections. Each institution is entitled to enter one participant in each contest. They must be student affiliate members. Complete rules for the contest are being sent to department heads. Those wishing to enter the contest must submit copies of their abstract to the Section Officers as outlined above. They should also include a letter indicating their desire to enter the contest. This letter should also be signed by the major Professor and the Department Head. A carbon copy of the letter and four additional copies of the abstract should be mailed to the contest representative.

For Production Section Award: Louis Boyd, Dairy Department, University of Tennessee, Knoxville

For Manufacturing Section Award: A. V. Moore, Department of Dairy Science, Texas A&M College, College Station

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

EXTENSION SECTION

Chairman: W. R. Van Sant, Department of Dairy Science, University of Arizona, Tucson.
Vice-Chairman: C. D. McGrew, Department of Dairy Science, Ohio State University, Columbus 10.
Secretary: D. E. Voelker, Department of Animal Husbandry, Iowa State University, Ames.

PRODUCTION SECTION

Chairman: J. C. Thompson, Ralston Purina Co., St. Louis, Missouri.
Vice-Chairman: L. H. Schultz, Department of Dairy Husbandry, University of Wisconsin, Madison.
Secretary: V. R. Smith, Department of Dairy Science, University of Arizona, Tucson.

MANUFACTURING SECTION

Chairman: M. L. Speck, Department of Food Science and Processing, North Carolina State College, Raleigh.

Vice-Chairman: D. M. Graham, Pet Milk Co., Research and Development Center, Greenville, Illinois.

Secretary: E. L. Thomas, Department of Dairy Industries, University of Minnesota, St. Paul.

(The Dairy Manufacturing Extension Section is a subsection of the Manufacturing Section. W. S. Arbuckle, Department of Dairy Husbandry, University of Maryland, College Park, is chairman, and A. L. Rippen, Department of Food Science, Michigan State University, East Lansing, is secretary. All abstracts for papers in this subsection should be submitted through the regular channels of the Manufacturing Section, but should be identified for presentation at the Dairy Manufacturing Extension Section meeting and a courtesy copy sent to W. S. Arbuckle.)

INVITATION FOR NOMINATIONS FOR VICE-PRESIDENT AND DIRECTORS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

A. C. Fay
Chairman, Nominating Committee,
12458 North Bay Shore Drive,
North Miami, Florida

Every member of the American Dairy Science Association is urgently requested to participate in the selection of officers and directors for the year 1962-63. In accordance with the By-Laws and established custom, and in order to maintain a balanced representation from the Sections, we are to elect this year:

(1) A Vice-President from the field of Dairy Manufacturing, who will automatically become President in the year 1963-64.

(2) One Director from the field of Dairy Production to serve for three years, and

(3) One Director from the field of Dairy Manufacturing to serve for three years.

As a member of A.D.S.A., you are specifically requested to submit your suggestions for each of these three important offices to the Chairman of the Nominating Committee before January 1, 1962, but preferably NOW, while it is fresh in your mind. In making these suggestions, consideration should be given to persons not only having inherent qualities of leadership but having demonstrated their devotion and interest in the affairs of the Association by serving as Sectional Officers or through membership on important committees. Experience in the business affairs of the Association is particularly important in your choice for the office of Vice-President.

On January 1, 1962, the Chairman of the Nominating Committee will assemble the suggestions received and request from some one of the nominators of each candidate a biographical sketch of his nominee for consideration of the members of the Committee. After selecting a slate of two candidates for each of the three offices and obtaining their acceptances, the list will be published in the 1962 April issue of the JOURNAL OF DAIRY SCIENCE, and later ballots will be submitted to the membership by the Secretary-Treasurer of the Association.

Members of the Nominating Committee are as follows:

Ralph E. Hodgson, USDA, Beltsville, Maryland
Marvin L. Speck, North Carolina State College, Raleigh, N.C.
J. C. Thompson, Ralston Purina Company, St. Louis, Missouri
W. R. VanSant, University of Arizona, Tucson, Arizona
A. C. Fay, 12458 North Bay Shore Drive, North Miami, Florida, Chairman.

Please give serious consideration to this request for your cooperation and act NOW by sending your suggestions to Chairman A. C. Fay.

PRICE SCHEDULE FOR REPRINTS OF PAPERS THAT APPEAR IN THE JOURNAL OF DAIRY SCIENCE

H. F. JUDKINS, Secretary-Treasurer
32 Ridgeway Circle, White Plains, New York

The Executive Board, at the time of the Annual Meeting of the American Dairy Science Association at the University of Wisconsin, increased the price of reprints 25%, effective July 1, 1961. The new reprint schedule follows:

No. of reprints	Number of pages								
	2	4	8	12	16	20	24	28	32
	(Cost in dollars)								
50	17.50	20.00	36.25	51.25	67.50	78.75	97.50	115.00	125.00
100	20.00	22.50	41.25	61.25	77.50	92.50	112.50	132.50	145.00
200	22.50	28.75	51.25	76.25	97.50	117.50	143.75	162.75	185.00
300	28.00	33.75	62.50	91.25	117.50	143.75	173.75	205.00	226.25
400	30.00	40.00	72.50	107.50	137.50	170.00	205.00	241.25	266.25
500	33.75	45.00	83.75	122.50	1575.0	195.00	236.25	277.25	306.25
600	37.50	51.25	93.75	137.50	177.50	221.25	266.25	313.75	346.25
700	41.25	56.25	105.00	153.75	197.50	246.25	297.50	350.00	387.50
800	45.00	62.50	115.00	168.75	218.75	272.50	328.75	386.25	427.50
900	48.75	67.50	126.25	185.00	238.75	298.75	358.75	422.50	467.50
1,000	57.25	73.75	136.25	200.00	258.75	323.75	390.00	458.75	507.50

If covers for reprints are desired, the cost of 50 covers will be $21.18, and for each additional 100 covers, the cost will be $8.75. Back copies of the JOURNAL will cost $2 each.

The reprints are made from standing type within 30 days after the papers appear in the JOURNAL. Requests for a few reprints of a paper should be sent to the authors, whose names and addresses appear with the title. The Secretary and the Editor's office do not keep supplies of the various reprints. Orders for large numbers of reprints should be sent to The Garrard Press, 510 North Hickory Street, Champaign, Illinois. These orders must be received within 30 days after the papers are published in the JOURNAL; otherwise, the type will have been destroyed.

In case the original type has been destroyed, it is possible to supply reprints by a special photographic process, and their cost will be 50% more than the regular ones. For example, 100 reprints of 32 pages will cost $217.50.

It is hoped that the publication of this reprint schedule will make it easier for interested people to obtain reprints in any number desired and, at the same time, aid in disseminating useful information to the dairy and related industries.

The JOURNAL OF DAIRY SCIENCE is copyrighted. Reproduction of complete papers from this JOURNAL by any organization is not permitted. The reproduction of graphs, tables, and illustrations for books and other periodicals may be authorized by the Editor-in-Chief.

BOOK REVIEW

MILK: THE MAMMARY GLAND AND ITS SECRETIONS. Ed., S. K. Kon and A. T. Cowie. Academic Press, New York and London. Volume I, 515 pp., $14 (100s); Volume II, 423 pp., $12 (86s). 1961.

These two volumes contain a comprehensive compilation of review articles prepared by a wide group of researchers concerned both directly and indirectly with studies on the mammary gland and/or its secretions.

Volume I contains four main sections: (1) The mammary gland, morphogenesis, structure, and growth, (2) functional physiology of the mammary gland, (3) lactation in man and farm animals, and (4) biochemical activities of the mammary gland. Twelve total review articles comprise this volume and include: Morphogenesis of the mammary gland, by Albert Raynaud; histology and cytology of the mammary gland, by Gaston Mayer and Marc Klein; hormonal regulation of mammary gland growth, by Dora Jacobsohn; the hormonal control of milk secretion, by A. T. Cowie; biochemistry of prolactin, by C. H. Li; neural control of lactation, by B. A. Cross; human lactation, by Michael Newton; farm animals: induction of lactation and galactopoiesis, by J. Meites; general biochemistry of mammary tissue, by R. G. Hansen and D. M. Carlson; protein metabolism, by J. M. Barry; the biosynthesis of lactose, by Luis F. Leloir and Carlos E. Cardini; and the biosynthesis of milk fat, by S. J. Folley and Mary L. McNaught.

Volume II contains two main sections entitled: (1) Nutrition of the lactating animal, and (2) nutritional value of milk. Eight total review articles comprise this volume and include: Nutrition of the lactating woman, by F. E. Hytten and A. M. Thomson; nutrition of lactating farm animals, by J. T. Reid; metabolic disturbances associated with lactation, by J. C. Shaw; dietary requirements for lactation in the rat and other laboratory animals, by Marjorie M. Nelson and H. M. Evans; the composition of milk and the nutritive value of its components, by E. R. Ling, S. K. Kon, and J. W. G. Porter; human milk and cow's milk in infant nutrition, by Icie G. Macy and Harriet J. Kelly; lactation and growth of the young, by K. L. Blaxter; and immunological aspects of colostrum, by R. Lovell and T. A. Rees. Each volume contains author and subject indices at the end, covering the contents in each respective volume. It would help somewhat if a cumulative index for both volumes had been included.

It will be readily appreciated from the above contents that these two volumes have been designed to provide an extensive coverage of the field. The quality of the authorship the editors have succeeded in obtaining to write the articles is also apparent. Although to some, specializing in certain areas of this field, it may seem that there may be an overbearing of authors from England, resulting in perhaps an emphasis being placed on the works of other local researchers to the partial exclusion of those located elsewhere geographically, over-all the editors have managed to obtain a fairly good international sprinkling of authorship. It is well to note that many of the contributors are not primarily members of the dairy science profession but are internationally recognized authorities in their respective basic fields of science; for example, C. H. Li writing on the biochemistry of prolactin, Luis F. Leloir and Carlos E. Cardini writing on lactose synthesis, etc. This helps to attest to the difficult job the editors have accomplished in assembling the works of such a distinguished set of authors.

As with all review articles, however, some authors have done a splendid job, but others have either not been so conscientious or have shown they are not completely qualified to write a critical review of their assigned area. Such articles appear to be in the minimum in these volumes. It is also unfortunate that such a lag has to occur between the writing of articles and their publication in book form. In these volumes, many references from 1958 are recorded, with an occasional one from early in 1959. Thus, the time lag is between two and three years.

In conclusion, it seems probable that any researcher connected with the field of dairy science will want ready access to these volumes. They should stand as one of the standard works in this field, both for the use of persons directly and indirectly concerned with the study of any aspect of the mammary gland and its secretions. On the whole, these two volumes probably represent as good a compilation of pertinent information on the mammary gland and its secretions as it is possible to accomplish.

B. L. LARSON
University of Illinois
Urbana, Illinois

DIFCO

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Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, *NINTH EDITION*,
including descriptions of these media and their use, is available on request.

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